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# 한국산 고라니(*Hydropotes inermis argyropus*)의 머리뼈 계측 및 분자유전학적 연구

# Craniometric and Molecular Genetic Characteristics of the Korean Water Deer, *Hydropotes inermis argyropus*

2014년 2월

서울대학교 대학원

수의학과 수의생리학/수의해부학 전공

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김영건

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A Dissertation for the Degree of Ph.D of Veterinary Physiology and Anatomy

# Craniometric and Molecular Genetic Characteristics of the Korean Water Deer, *Hydropotes inermis argyropus*

Advised by Professor Hang LEE and Professor Junpei KIMURA

By Yung-Kun KIM

A Dissertation Submitted to the Faculty of the Graduate School of Seoul National University in Partial Fulfillment of the Requirements for the Degree of Ph. D. of Veterinary Physiology and Anatomy

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# Craniometric and Molecular Genetic Characteristics of the Korean Water Deer, *Hydropotes inermis argyropus*

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## ABSTRACT

The water deer, *Hydropotes inermis*, is the only species of genus *Hydropotes* and has conventionally been classified into two

subspecies according to geographic distribution and pelage color pattern: *H. i. inermis* from China and *H. i. argyropus* from Korea. Contrasted with the Chinese water deer, the Korean subspecies has been rarely studied. In this dissertation, some morphological and genetic characteristics of the Korean water deer populations were investigated. This volume consists of two parts (Chapter I for morphological and Chapter II for genetic studies).

In Chapter I, three different topics were executed. First, the skull growth of the Korean water deer was analyzed using craniometric method. Second, the sexual dimorphism of skull of the Korean water deer was evaluated using cranio-mandibular analysis. Third, the morphological differences of crania between two subspecies of water deer (*H. i. inermis* and *H. i. argyropus*) were determined, based on the cranial measurements.

In Chapter II, two different topics in one chapter were studied. First, mitochondrial cytochrome *b* gene diversity and phylogeny of the Korean water deer were examined. Second, population structure of

the Korean water deer was analyzed.

For the first topic, craniomandibular traits of the water deer from the Korean Peninsula were examined to assess size change in growth between age groups and sexes. Univariate and multivariate analyses were conducted based on 34 cranial and 11 mandibular measurements from both sexes. Statistical comparisons of skull measurements revealed a significantly different growth pattern between the sexes. For the male, the size change of the cranium and mandible was straight through age groups, constantly. On the other hand, the size of the cranium and mandible of the female was changed relatively steeper than that of the male in age groups 2 to 3, and the growth curves from age group 3 to 4 were more gradual than age groups 2 to 3. Principal component analysis showed that these 2 sexes have a similar trend. In the allometry analysis, there were differences in growth in 5 traits in both sexes. In conclusion, my study suggests that the male and the female Korean water deer had a similar trend for their growth, although there was a small difference

of skull growth for age groups.

For the second topic, Sexual dimorphism in the craniomandibular traits in the Korean water deer was examined for the first time. Multivariate analyses using only cranial traits showed a clear separation between sexes. However, the separation was not obvious in the discriminant analysis using only mandibular traits. The most clearly dimorphic trait was in the incisive bone breadth, which was about 12% larger in males. The incisive bone breadth reflects the characteristically large canines in male. In contrast to this, most of the cranial measurements, except for the incisive breadth, were larger in female, indicating a larger overall skull size. Given that males are generally larger than females, this sexually dimorphic pattern is unique among mammals. I propose that factors, for example, a unique parental care, have influenced the larger skull size in the females of this species.

The water deer has conventionally been classified into two subspecies according to geographic distribution and pelage color

pattern: Chinese water deer from China, and Korean water deer from Korea. However, the results of a recent molecular study have called this into question. To further reappraise this classification, I examined morphological variation in craniodental measurements of these two subspecies. Results of Student's *t*-test analysis and multivariate analyses demonstrated that these two subspecies are not well-differentiated, suggesting that individuals of the two populations share common morphological traits. Despite the distribution of the subspecies at different latitudes, no clear morphocline was detected suggesting that Bergmann's rule does not apply in this case. Discriminant analysis indicated that the characteristics of some individuals are shared by both populations, suggesting that not all individuals can be assigned to their original population. Results of principal component analysis showed that the two populations shared more than 75% of individuals, congruent with the "75% rule" of subspecies classification. In both the neighbor-joining and unweighted pair group methods with arithmetic mean cluster analyses, specimens of *H. i. argyropus* and

*H. i. inermis* were highly mixed within the cladograms. These results suggest that the overall morphological variation in the two subspecies overlaps considerably and that there is no coherent craniodental difference between the two groups. The present findings combined with prior observations from molecular biogeography point out that the taxonomic division of water deer into two subspecies should be revisited.

For the fourth topic, we analyzed 1,140 base pair of mtDNA cytochrome *b* gene and 12 microsatellite markers of the Korean water deer, respectively. Twenty-one haplotypes were detected in 51 samples from three regional populations in the Korea, and the overall genetic diversity was relatively low compared to the Chinese water deer, sister taxa of the Korean water deer. Although phylogenetic analysis revealed that there are two distinct clades, the regional division was not detected. Microsatellite variability was not significant among three populations (mean $F_{ST}$ = 0.008) and it means that there is no population structure. These results suggest

that the Korean water deer populations show a single population although there were two distinct clades. As basic, essential and useful information for animal research, this data will be helpful for conservation.

These results from the four topics in Chapter I and II show that these fundamental and integrative studies of the Korean water deer will be useful to understand the morphological and genetic characteristics, and furthermore to perform its conservation and management strategy in Korea and China.

**KEY WORDS:** Craniomandibular Morphology, Cytochrome *b* Gene, Genetic Diversity, Growth Pattern, *Hydropotes inermis argyropus*, Korean Water Deer, Microsatellite Marker, Mitochondrial DNA, Population Structure, Sexual Dimorphism

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# TABLE OF CONTENTS

# LIST OF TABLES

# LIST OF FIGURES

# GENERAL INTRODUCTION

**Origin of the name "Water Deer"**

In Korea, "Water deer" is called as "고라니(Gorani)", "보노루(Bonoru)"or "복작노루(Bokjaknoru)" (윤 등., 1967). However, what is the origin of this name is still unknown. In China, 狍 (páo), the Chinese character, means "water deer." In Japan, "キバノロ (Kibanoro)" is used as the name of "Water deer." "キバ(Kiba)" means Canines and "ノロ(Noro)" means deer.

The meaning of *Hydropotes inermis*, the scientific name of Water deer, is "living near riverside" for *Hydropotes* and "animal that do not have weapon" for *inermis*. Thus, the ecological characteristics of water deer can be imagined by their name (Lee, 2003).

Chinese water deer had been reported in 1870 for the first time by Swinhoe, and this species was investigated near the lower Yangtze Basin (Swinhoe, 1870). Maybe, he thought water deer roaming riverside is familiar with water and gave that name to this species. Korean water deer, *H. i. argyropus*, the subspecies of water deer, was reported at the first time by Heude after 14 years the Chinese water deer had been reported (Heude, 1884).

**Taxonomy of Water Deer (*Hydropotes inermis*)**

According to traditional classification, Groves and Grubb (1987) divided the Cervidae into three different subfamilies: Hydropotinae, Odocoileinae and Cervinae. Among these, the water deer is the only species in the genus *Hydropotes*, subfamily Hydropotinae, family Cervidae: *Hydropotes inermis* (Putman, 1988). Two subspecies of water deer have traditionally been recognized. One is the Chinese water deer (*Hydropotes inermis inermis*, Swinhoe 1870), distributed in the lower Yangtze Basin, west to Hupeh in China. The other is the

Korean water deer (*Hydropotes inermis argyropus*, Heude 1884), distributed throughout the whole of the Korean Peninsula (Allen, 1940).

According to the recent study using 2 mitochondrial protein-coding gene (cytochrome *b* and CO2) and 2 nuclear fragments (intron 2 of $\alpha$-lactalbumin and intron 1 of the gene encoding the protein kinase C iota), the result presented a new classification of family Cervidae (Gilbert et al., 2006).

In a new classification, family Cervidae consists of 2 subfamilies, Cervinae and Capreolinae, and each subfamily is composed 2 tribes and 3 tribes, respectively. Cervinae and Capreolinae shows difference in the metacarpals: (1) Cervinae (= Plesiometacarpalia) unites Cervinae and Muntiacini, (2) Capreolinae (= Telemetacarpalia) includes Alceini, Capreolini and Odocoileini. This study shows that water deer is belonged to tribe Capreolini.

**Morphology**

Water deer is actually very similar in appearance to musk deer (Putman, 1998). Its prominent tusks have led to it being colloquially named the vampire deer in English-speaking areas to which it has been imported. Despite its lack of antlers and certain anatomical peculiarities, including a pair of prominent tusks in male, it is classified as a cervid.

Like other deer, water deer does not carry a gallbladder and nor a spiral urethral terminus on the penis. Juvenile of this species are spotted on the back in the manner of deer, not in the manner of tragulids. Additionally, water deer has small preorbital glands, interdigital glands on the hind legs and a pair of inguinal glands, the only cervid to have such (Allen, 1940).

Its legs are relatively long and slender, in particular the metacarpals (Scott, 1987), and well-suited for fast running to escape their many predators. During evolutionary time, the leg bones of deer became longer and the weight of the animal became supported entirely on

the third and fourth toes, eventually resulting in the evolution of cloven hooves. The second and fifth toes are short and positioned up, as dewclaws. The first digit has vanished and the bones of the palm (metacarpals and metatarsals) have been forged into a single bone, the cannon bone.

There are three kinds of reduction type of frontal leg metapodia (Geist, 1998): (1) The ***holometacarpalian*** condition, as found in pigs. The second and fifth metapodia are still intact, although the first metapodium is missing. (2) The ***telemetacapalian*** condition, as found in New World deer, musk deer, water deer and various extinct ruminant families. The second and fifth metapodia have been reduced to distal splinters. (3) The ***plesiometacarpalian*** condition, as typically found in Old World deer. The second and fifth metapodia are reduced to their proximal splinters.

**Population Genetic Studies**

For Chinese water deer, Hu et al. (2006 and 2007) examined the genetic diversity and population structure by analyzing 403 base pairs fragment of the mitochondrial DNA control region and the nuclear genetic diversity and population structure by using 7 microsatellite markers, respectively.

In the study using mitochondrial DNA control region, they detected 18 different haplotypes from 40 specimens of two different populations (one is from Zhoushan archipelago, and the other is from mainland), and the genetic diversity was relatively higher than other cervid species. Haplotype diversity was 0.923±0.025 and nucleotide diversity was 1.318±0.146%. In this study, analysis of molecular variance (AMOVA) showed significant differentiation between two populations and it suggests there is no individual exchange between Zhoushan and the mainland population.

In the study using 7 microsatellite markers, two representative

captive populations with different origin were used with a Zhoushan archipelago wild population for control. The mean number of observed alleles per locus was 5.143, and the average observed and expected heterozygosity values were 0.662 and 0.531, respectively. And also, strong differentiation between them was detected.

In the case of the Korean water deer, genetic study has not been carried out, however recent studies examined taxonomic status of the Korean water deer and compared with the Chinese water deer. Koh et al. (2009) obtained 927 base pairs of partial mitochondrial DNA (mtDNA) control region and 1,140 base pairs of cytochrome *b* gene full sequences of water deer from China and Korea to examine the taxonomic status of two subspecies. In this study, they found that there are two sympatric mtDNA clades (a major clade from China and Korea and a minor clade from Korea). The average genetic distance was 2.1% in the control region and 1.3% in the cytochrome *b* gene. They discussed that current taxonomic status of water deer is not consistent with their finding and it is needed to reconsider

classification of water deer, using not only nuclear DNA marker analyses but also morphological characters.

**Purpose of this Thesis**

As mentioned above, some studies of the Korean water deer have been reported in various fields. In this dissertation, there are two major topics: First is to investigate morphological characteristics (for example, growth pattern, sexual dimorphism and geographical difference) of the water deer. Second is to show molecular genetic characteristics (for example, genetic diversity and population structure) of the Korean water deer.

# CHAPTER I: Morphological studies of the Korean water deer, *Hydropotes inermis argyropus*

## Part I. Skull Growth of the Korean Water Deer, *Hydropotes inermis argyropus*

### INTRODUCTION

The skull of the vertebrate basically protects the brain and sensory organs, and additionally associates with masticatory movement and locomotion. It means that the skull has several roles as multiple functional and developmental units. Postnatal ontogeny and the size of the skull can be influenced by several environmental and genetic factors (Burnett, 1983; Hall, 1989; Limborgh, 1972; Wiggington and Dobson, 1999; Yom-Tov and Nix, 1986). As a functional and developmental unit, the skull of an animal can be useful not only for classification of taxa, but also for the study of life history strategies

and evolutionary change in animal species (Lu, 2003).

The water deer, *Hydropotes inermis*, is one species of genus *Hydropotes* and distributed in the China and the Korean Peninsula (Allen, 1940; Geist, 1998). Two subspecies of water deer are reported, Korean water deer, *H. i. argyropus* and Chinese water deer, *H. i. inermis*. Originally, the habitats of this species had stretched from the eastern part of China to the Korean Peninsula, but the current habitats are divided between China and Korea, and even its habitats are isolated within China.

There are some anatomical studies of this species which include the male genital organ, the rumen structure and the branching pattern of the aortic arch (Ahn et al., 2008; Hofmann et al., 1988; Sohn and Kimura, 2013). However, there is no study which has analyzed the skull growth, sexual dimorphism and geographical difference of water deer. In this dissertation, I compared the skull growth between the sexes and among four age groups which were defined by the degree of molar eruption, and I analyzed sexual dimorphism using

craniomandibular traits. Additionally, I compared skull morphological traits between two subspecies of water deer to produce evidence for classification status of water deer. This is the first study to examine the morphology including growth pattern, sexual dimorphism and geographical difference of *H. inermis* and will be helpful to the understanding of factors related to the growth and evolution of this species.

## MATERIALS AND METHODS

A total of 96 crania and 92 mandibles of Korean water deer were examined in this study. Skull specimens were prepared from the carcasses found in Korea and then stored in the Department of Anatomy and Cell Biology, College of Veterinary Medicine, Seoul National University, Seoul, Korea. Based on Driesch's method (Driesch, 1976), 34 cranial and 11 mandibular measurements were taken to the nearest 0.1 mm and recorded as summarized in Table 1. Linear measurements are illustrated in Fig. 1. As the length dimension of cranium, 19 variables (TL, CBL, BL, SSL, PPL, BCL, BFL, NCL, MFL, LNL, LRL, LPL, AIOL, NL, SL, MPL, OPL, LIL and VCL) were measured. As the breadth measurements of cranium, 10 variables (FMB, IB, LFB, LOB, MB, NB, OB, OCB, POPB and ZB) were measured. For mandible, categories can be divided into two different groups; 6 variables for length dimension (MLA, MLC, Gp2L, GDFL, LPRL and DL) and 5 variables for height dimension (AVRH, MVRH, OVRH, MH1 and MH2).

To identify growth patterns, four age groups were determined by the state of eruption and attrition of the molars (Table 2). Using teeth for age determination is commonly reported in many species of deer (Lowe, 1967; Mitchell, 1963, 1967; Sergeant and Pimlott, 1959; Severinghaus, 1949; Terai et al., 1998). This method is based on eruption sequence, wearing estimation and cementum lines (Morris, 1972). All individuals were classified into four age groups, and information of each age group is shown in Table 2. Sex was determined by observing genital organs.

Two different statistical tests were performed, Student's *t*-test on the sexes for difference and analysis of variance (ANOVA) among age groups using PASW Statistics v18 program (IBM Acquires SPSS Inc., Chicago, IL, U.S.A). In multivariate treatments, I conducted principal component analysis (PCA) based on variance-covariance matrix of cranium and mandible, separately. PCA was conducted with the software PAST version 2.07 (Hammer et al., 2001).

In addition to the above analyses, I performed allometric analyses.

To describe patterns of shape change accompanied by growth, allometry has often been employed (Abdala, 2001; Gianninni et al., 2004; Gianninni et al., 2010; Kurihana and Oda, 2009). I used bivariate allometry for each trait for each sex. The bivariate allometry equation is expressed as following: $log\ y=\alpha \cdot log\ x + \beta$, which is the common logarithmic conversion of the formula $y=b \cdot x^{\alpha}$ ($\beta=log\ b$), where $\alpha$ is allometry coefficient (equilibrium constant) and $\beta$ is the initial growth index (Huxley and Tessier, 1936). I compared $\alpha$ and $\beta$ of allometry equations between sexes using TL as an independent variable. The allometry coefficient was estimated by standardized major axis (often called as reduced major axis) (Warton et al., 2006). Test of allometry (whether $\alpha$ is different from 1.0) and the comparison of the 2 parameters between sexes were conducted by using SMATR package (Warton and Ormerod, 2005) for the software R (R Development Core Team, 2011).

Table 1. List of the cranium and mandible measurements of the water deer used in this study.

| Category | Acronym | Measurement |
|---|---|---|
| CRANIUM | TL | Total length |
| | CBL | Condylobasal length |
| | BL | Basal length |
| | SSL | Short skull length |
| | PPL | Premolare - Prosthion |
| | BCL | Basicranial axis |
| | BFL | Basifacial axis |
| | NCL | Neurocranium length |
| | VCL | Viscerocranium length |
| | MFL | Median frontal length |
| | LNL | Lambda - Nasion |
| | LRL | Lambda - Rhinion |
| | LPL | Lambda - Prosthion |
| | AIOL | Akrokranion - Infraorbitale of one side |
| | NL | Greatest length of the nasals |
| | SL | Snout length |
| | MPL | Median palatal length |
| | OPL | Oral palatal length |
| | LIL | Lateral length of the incisive bone |
| | UPRL | Length of the upper premolar row |
| | LO1 | Length of the orbit (upper suture) |
| | LO2 | Length of the orbit (lower suture) |
| | MB | Greatest mastoid breadth |

Table 1. continued.

| | | |
|---|---|---|
| | OCB | Greatest breadth of the occipital condyles |
| | POPB | Greatest breadth at the bases of the paraoccipital processes |
| | FMB | Greatest breadth of the foramen magnum |
| | FMH | Greatest height of the foramen magnum |
| | LFB | Least frontal breadth |
| | ZB | Zygomatic breadth |
| | LOB | Least breadth between the orbits |
| | OB | Greatest breadth across the orbits |
| | NB | Greatest breadth across the nasals |
| | IB | Greatest breadth across the incisive bone |
| | BNCH | Basion - the highest point of the superior nuchal crest |
| MANDIBLE | MLA | Mandible length from the angle |
| | MLC | Mandible length from the condyle |
| | Gp2L | Gonioncaudale – oral border of the alveleolus of p2 |
| | GDFL | Gonioncaudale – the most aboral indentation of the dental foramen |
| | LPRL | Length of the lower premolar row |
| | DL | Length of the diastema |
| | AVRH | Aboral height of the vertical ramus |
| | MVRH | Middle height of the vertical ramus |
| | OVRH | Oral height of the vertical ramus |
| | MH1 | Height of the mandible in front of m1 |
| | MH2 | Height of the mandible in front of p2 |

Table 2. Information of each age group.

| Age group | Male | | Female | | Fully erupted molars | Attrited molars |
|---|---|---|---|---|---|---|
| | Cranium | Mandible | Cranium | Mandible | | |
| I | 5 | 4 | 9 | 9 | M1 | - |
| II | 11 | 11 | 12 | 12 | M1 and M2 | - |
| III | 31 | 27 | 15 | 15 | M1, M2 and M3 | - |
| IV | 6 | 7 | 7 | 7 | M1, M2 and M3 | M1, M2 and M3 |
| Total | 53 | 49 | 43 | 43 | | |

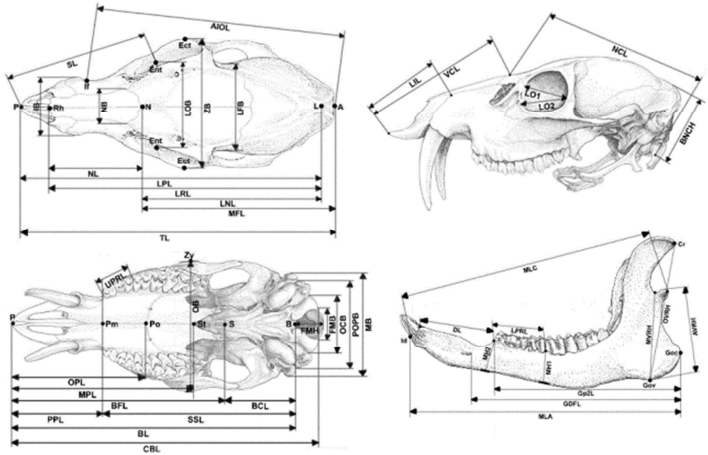


Fig. 1. Craniofacial measurements of the water deer used in this study. Abbreviations are given in Table 1. (A: Akrokranion, B: Basion, Cr: Coronion, Ect: Ectorbitale, Ent: Entorbitale, Goc: Gonion caudale, Gov: Gonion ventral, Id: Infradentale, If: Infraorbitale, L: Lambda, N: Nasion, P: Prosthion, Pm: Premolare, Po: Palatinoorale, Rh: Rhinion, S: Synsphenion, St: Staphylion and Zy: Zygion).

## RESULTS

*Univariate analysis*: Among 19 cranial traits which represent length dimension, all traits except 4 variables (PPL, LNL, NL and OPL) showed no significant difference in the female between the age groups 3 and 4. There was a significant difference in all age group pairs in PPL and OPL. In some age groups of LNL and NL, significant differences were found. Although the size of UPRL did not show significant difference in some age groups, the size of UPRL decreased in age groups 1 to 4 in both sexes. In the height measurements of cranium, 2 variables (FMH and BNCH) were measured. Although significant differences could not be confirmed in age groups 1 to 4 in both sexes, the size of FMH decreased (Table 3). The size of BNCH increased throughout all age groups, although there were no significant differences in some age groups. The size of 2 variables which are related to the size of the eye (LO1 and LO2) significantly increased in age groups 1 to 4.

Table 3. Descriptive statistics and results of ANOVA test of the cranial variables in each sex and age groups of the Korean water deer

| Variable | Sex | Age groups | | | | ANOVA test | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | Age group 1 vs 2 | Age group 1 vs 3 | Age group 1 vs 4 | Age group 2 vs 3 | Age group 2 vs 4 | Age group 3 vs 4 |
| TL | M | 150.11 | 158.90 | 167.06[c] | 175.18 | ** | *** | *** | *** | *** | *** |
| | F | 151.88 | 159.95 | 172.84 | 175.68 | ** | *** | *** | *** | *** | ns |
| CBL | M | 139.23 | 149.52 | 156.62[c] | 164.16 | *** | *** | *** | *** | *** | *** |
| | F | 140.07 | 149.26 | 162.40 | 166.27 | *** | *** | *** | *** | *** | ns |
| BL | M | 128.80 | 139.30 | 146.47[c] | 154.01 | *** | *** | *** | *** | *** | *** |
| | F | 130.05 | 139.14 | 152.30 | 156.06 | *** | *** | *** | *** | *** | ns |
| SSL | M | 82.95 | 89.36 | 93.97[c] | 96.33 | *** | *** | *** | *** | *** | * |
| | F | 84.67 | 90.22 | 97.53 | 97.83 | *** | *** | *** | *** | *** | ns |
| PPL | M | 45.93 | 50.03 | 52.45[b] | 57.67 | ** | *** | *** | ** | *** | *** |
| | F | 45.65 | 48.93 | 54.77 | 58.05 | ** | *** | *** | *** | *** | ** |
| BCL | M | 32.79 | 34.91 | 36.53 | 38.03 | * | *** | *** | ** | *** | * |
| | F | 33.74 | 34.94 | 37.34 | 38.60 | ns | *** | *** | *** | *** | ns |
| BFL | M | 98.35 | 106.55 | 112.14[c] | 118.02 | *** | *** | *** | *** | *** | *** |
| | F | 99.21 | 106.50 | 117.03 | 119.12 | *** | *** | *** | *** | *** | ns |

Table 3. continued.

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| NCL | M | 85.98 | 89.26 | 92.45[a] | 95.50 | * | *** | *** | *** | *** | ** |
| | F | 86.84 | 90.29 | 94.56 | 96.21 | * | *** | *** | ** | *** | ns |
| VCL | M | 70.26 | 76.63 | 80.59[c] | 85.99 | ** | *** | *** | ** | *** | ** |
| | F | 70.96 | 76.33 | 84.92 | 84.80 | ** | *** | *** | *** | *** | ns |
| MFL | M | 86.96 | 88.34 | 93.10[a] | 96.51 | ns | *** | *** | *** | *** | ** |
| | F | 88.33 | 90.76 | 94.96 | 96.72 | ns | *** | *** | ** | ** | ns |
| LNL | M | 78.03 | 78.80 | 82.45 | 84.67 | ns | ** | *** | *** | *** | ns |
| | F | 78.62 | 80.46 | 83.60 | 86.05 | ns | ** | *** | * | ** | ns |
| LRL | M | 122.85 | 127.84 | 134.30[b] | 138.83 | * | *** | *** | *** | *** | * |
| | F | 121.94 | 128.36 | 138.17 | 138.92 | ** | *** | *** | *** | *** | ns |
| LPL | M | 143.93 | 151.90 | 159.31[c] | 166.61 | ** | *** | *** | *** | *** | ** |
| | F | 145.15 | 152.59 | 164.56 | 167.68 | ** | *** | *** | *** | *** | ns |
| AIOL | M | 106.35 | 111.24 | 116.59[c] | 121.01 | ** | *** | *** | *** | *** | ** |
| | F | 107.74 | 112.49 | 120.07 | 121.63 | ** | *** | *** | *** | *** | ns |
| NL | M | 46.13 | 49.83 | 52.46[a] | 55.01 | ns | ** | ** | ns | * | ns |
| | F | 44.84 | 48.93 | 55.29 | 53.25 | * | *** | *** | *** | * | ns |
| SL | M | 69.26 | 75.23 | 79.95[c] | 84.41 | ** | *** | *** | *** | *** | ** |
| | F | 69.81 | 75.63 | 84.01 | 85.24 | *** | *** | *** | *** | *** | ns |
| MPL | M | 81.14 | 90.34 | 94.65[c] | 100.87 | *** | *** | *** | ** | *** | *** |
| | F | 83.04 | 89.68 | 99.39 | 102.18 | *** | *** | *** | *** | *** | ns |
| OPL | M | 61.86 | 68.02 | 71.58[b] | 76.00 | ** | *** | *** | ** | *** | ** |
| | F | 62.51 | 67.73 | 74.44 | 77.54 | *** | *** | *** | *** | *** | * |

Table 3. continued.

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| LIL | M | 40.59 | 44.01 | 46.35[a] | 49.19 | * | *** | *** | * | ** | * |
| | F | 41.21 | 43.58 | 48.62 | 49.69 | ns | *** | *** | *** | *** | ns |
| UPRL | M | 24.61 | 24.45[a] | 24.21 | 22.44 | ns | ns | ** | ns | ** | ** |
| | F | 25.31 | 25.33 | 23.88 | 22.67 | ns | * | *** | ** | *** | ns |
| LO1 | M | 23.83 | 24.44 | 25.31 | 26.47 | ns | ** | *** | * | *** | ** |
| | F | 24.07 | 24.55 | 25.62 | 26.72 | ns | *** | *** | ** | *** | * |
| LO2 | M | 23.59 | 24.70 | 24.88 | 26.33 | * | ** | *** | ns | ** | ** |
| | F | 23.43 | 24.10 | 25.14 | 26.21 | ns | ** | *** | * | *** | * |
| MB | M | 42.99 | 45.87 | 47.02 | 48.61 | ** | *** | *** | * | ** | * |
| | F | 44.38 | 45.22 | 47.17 | 48.36 | ns | ** | *** | * | ** | ns |
| OCB | M | 27.07 | 29.82b | 28.73 | 30.31 | *** | * | *** | * | ns | * |
| | F | 28.64 | 28.44 | 29.28 | 29.28 | ns | ns | ns | * | ns | ns |
| POPB | M | 38.37[a] | 41.42 | 41.96 | 43.86 | ** | *** | *** | ns | ** | ** |
| | F | 40.11 | 40.64 | 41.84 | 42.87 | ns | * | ** | ns | ** | ns |
| FMB | M | 13.15[a] | 14.41 | 14.13 | 14.62 | * | * | ** | ns | ns | ns |
| | F | 14.30 | 14.26 | 14.02 | 14.43 | ns | ns | ns | ns | ns | ns |
| FMH | M | 15.63 | 15.20 | 14.81 | 14.97 | ns | ns | ns | ns | ns | ns |
| | F | 15.16 | 15.31 | 14.76 | 14.60 | ns | ns | ns | ns | ns | ns |
| LFB | M | 37.34 | 36.88 | 37.80 | 37.89 | ns | ns | ns | * | ns | ns |
| | F | 37.23 | 37.33 | 38.37 | 36.91 | ns | ns | ns | ns | ns | * |
| ZB | M | 65.88 | 69.76 | 71.15 | 74.78 | ** | *** | *** | ns | *** | ** |
| | F | 66.78 | 69.20 | 72.34 | 76.35 | * | *** | *** | ** | *** | *** |

Table 3. continued.

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| LOB | M | 35.94 | 36.37[a] | 39.44 | 40.83 | ns | ** | ** | ** | ** | ns |
| | F | 36.97 | 38.79 | 40.60 | 39.96 | ns | ** | * | ns | ns | ns |
| OB | M | 63.44 | 68.65 | 70.79 | 73.08 | *** | *** | *** | ** | *** | * |
| | F | 64.56 | 67.88 | 71.67 | 73.54 | ** | *** | *** | ** | *** | ns |
| NB | M | 14.72 | 14.79 | 16.11 | 17.40 | ns | ns | ** | * | ** | ns |
| | F | 14.65 | 15.33 | 16.01 | 17.47 | ns | * | ** | ns | ** | * |
| IB | M | 24.09 | 26.97[b] | 29.48[c] | 33.19[c] | * | *** | *** | *** | *** | ** |
| | F | 22.82 | 23.90 | 25.97 | 28.02 | ns | *** | *** | *** | *** | ** |
| BNCH | M | 40.16 | 41.27 | 41.33[a] | 42.75 | ns | * | ** | ns | * | ** |
| | F | 40.28 | 41.04 | 42.38 | 42.50 | ns | ** | ** | * | * | ns |

Significant difference between the male and the female: [a] $p < 0.05$, [b] $p < 0.01$ and [c] $p < 0.001$, and between each age group: ns $p > 0.05$, * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

While the 6 length dimensions of the mandible showed similar growth patterns in both sexes, Gp2L did not show a significant difference between age groups 3 and 4. In the cases of DL and LPRL, significant differences were not found when comparing age groups 1 and 2. Among 5 height factors, the growth of MH1 in the male was not found. Growth in age groups 1 to 4 in other factors was found partially or through all age groups.

Mean values of cranial traits were different between sexes in 2 variables (POPB and FMB) in age group 1; 3 variables (OCB, LOB and IB) in age group 2; 19 variables (TL, CBL, BL, SSL, PPL, BFL, NCL, VCL, MFL, LRL, LPL, AIOL, NL, SL, MPL, OPL, LIL, IB and BNCH) in age group 3 and 1 variable (IB) in age group 4 (Table 3). Except for OCB and IB, all traits were larger in the female specimens. IB is the most notable trait measurement in both sexes, and is related to the upper canines. Canines developed in the male (Fig. 2A, arrow), but poorly developed in the female (Fig. 2B, arrow).

Mean values of mandibular traits were different in both sexes, with 1 variable (MH2) in age group 2 and 4 variables (MLA, MLC, Gp2L and GDFL) in age group 3 (Table 4). All these 5 traits were larger in the female.

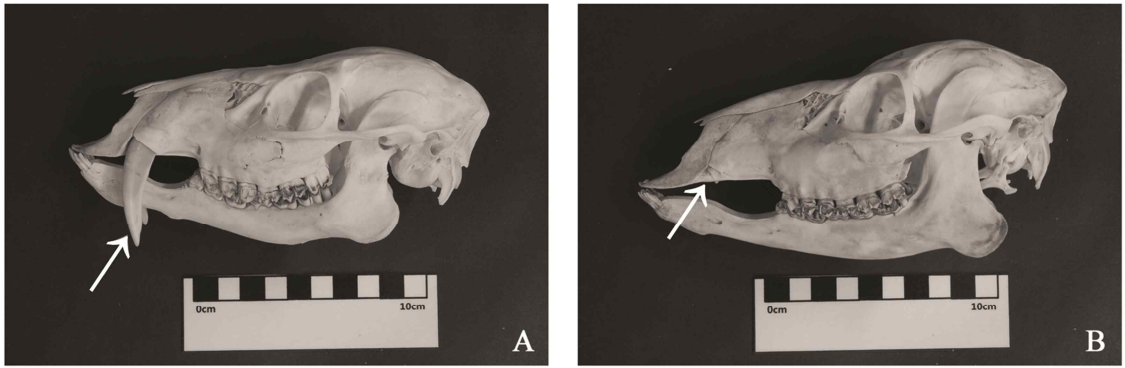


Fig. 2. Skull of the Korean water deer. A) Male (Specimen ID: KJ0163, age group 3), left lateral view. B) Female (Specimen ID: KJ0138, age group 3), left lateral view. Arrows: canines.

Table 4. Descriptive statistics and results of ANOVA test of the mandibular variables in each sex and age groups of the Korean water deer

| Variable | Sex | Age groups | | | | ANOVA test | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | I | II | III | IV | Age group 1 vs 2 | Age group 1 vs 3 | Age group 1 vs 4 | Age group 2 vs 3 | Age group 2 vs 4 | Age group 3 vs 4 |
| MLA | M | 117.50 | 128.86 | 136.42[a] | 142.54 | *** | *** | *** | *** | *** | ** |
| | F | 118.83 | 127.48 | 139.18 | 143.23 | *** | *** | *** | *** | *** | * |
| MLC | M | 115.33 | 123.05 | 130.27[a] | 136.45 | ** | *** | *** | *** | *** | *** |
| | F | 115.47 | 123.17 | 133.44 | 137.81 | *** | *** | *** | *** | *** | * |
| Gp2L | M | 76.28 | 84.86 | 89.13[b] | 91.11 | *** | *** | *** | *** | *** | ns |
| | F | 78.07 | 84.50 | 91.61 | 92.82 | *** | *** | *** | *** | *** | ns |
| GDFL | M | 91.75 | 99.73 | 105.75[b] | 109.19 | ** | *** | *** | *** | *** | * |
| | F | 94.42 | 99.66 | 109.44 | 110.63 | ** | *** | *** | *** | *** | ns |
| LPRL | M | 25.49 | 26.09 | 24.57 | 20.82 | ns | ns | *** | * | *** | *** |
| | F | 26.59 | 26.72 | 24.12 | 21.42 | ns | * | *** | ** | *** | ** |
| DL | M | 35.99 | 38.05 | 41.68 | 46.80 | ns | *** | *** | *** | *** | *** |
| | F | 35.33 | 36.42 | 41.89 | 45.01 | ns | *** | *** | *** | *** | ** |
| AVRH | M | 36.77 | 38.48 | 40.77 | 43.05 | ns | *** | *** | *** | *** | ** |
| | F | 35.29 | 39.10 | 41.68 | 43.90 | *** | *** | *** | ** | *** | * |
| MVRH | M | 34.57 | 36.91 | 38.75 | 40.86 | * | *** | *** | ** | *** | ** |
| | F | 33.76 | 37.39 | 39.81 | 40.81 | ** | *** | *** | ** | ** | ns |

Table 4. continued.

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| OVRH | M | 55.15 | 61.59 | 63.10 | 65.86 | ** | *** | *** | ns | ** | * |
| | F | 54.84 | 60.90 | 64.71 | 66.79 | ** | *** | *** | * | ** | ns |
| MH1 | M | 13.22 | 14.30 | 14.33 | 13.61 | ns | ns | ns | ns | ns | ns |
| | F | 12.99 | 14.52 | 15.04 | 14.84 | ** | *** | ** | ns | ns | ns |
| MH2 | M | 11.69 | 12.71[a] | 14.02 | 13.77 | ns | *** | ** | ** | * | ns |
| | F | 11.68 | 13.41 | 14.21 | 14.53 | *** | *** | *** | * | ** | ns |

Significant difference between the male and the female: [a] $p < 0.05$, [b] $p < 0.01$ and [c] $p < 0.001$, and between each age group: ns $p > 0.05$, * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

*PCA*: The first 5 principal components which account for more than 1 value of eigenvalue explained 62.71, 5.46, 4.69, 3.95 and 3.46% of total variation, respectively and heavy loads for 29 traits, except for 5 traits (UPRL, OCB, FMB, FMH and LFB) in the cranium (Table 5). Factor loadings for PC1 were all positive except for 2 dimensions (UPRL and FMH) and ranged from 0.983 in TL to 0.121 in FMB. Among the 5 traits, 3 traits (OCB, FMB and FMH) were attributed to PC2. Factor loadings for PC2 were the largest in FMB (0.655), FMH (0.572) and OCB (0.517). The first 2 principal components which account for more than 1 value of eigenvalue explained 74.01 and 10.06% on the mandible, respectively (Table 6). It is shown that 9 variables attributed to PC1, except for MH1 (−0.033) and MH2 (0.468). Among the 9 variables, LRPL (−0.892) is negatively correlated with PC1. Except for LRPL (0.097) and DL (0.331), 9 variables are attributed to PC2. In plots between PC1 and PC2 of the cranium and mandible, samples from the male from all age groups as well as those from the female overlapped partially with neighboring age groups, except for 3 cases. Scatter pattern

between age groups 1 and 2, age groups 3 and 4 of the male mandible and age groups 2 and 3 of the female mandible did not overlap with each other (Fig. 3).

Table 5. Principal components of the cranium which account for more than 1 of eigenvalue from PCA.

| Variable | Cranium | | | | |
|---|---|---|---|---|---|
| | PC1 | PC2 | PC3 | PC4 | PC5 |
| TL | **0.983** | -0.058 | 0.073 | 0.108 | -0.010 |
| CBL | **0.980** | -0.118 | 0.078 | 0.015 | 0.038 |
| BL | **0.976** | -0.148 | 0.067 | -0.013 | 0.043 |
| SSL | **0.928** | -0.185 | -0.003 | 0.012 | 0.157 |
| PPL | **0.932** | -0.082 | 0.147 | -0.034 | -0.085 |
| BCL | **0.786** | -0.158 | -0.148 | -0.082 | 0.279 |
| BFL | **0.968** | -0.124 | 0.128 | 0.044 | -0.017 |
| NCL | **0.862** | 0.073 | -0.268 | 0.173 | 0.151 |
| VCL | **0.921** | -0.155 | 0.295 | 0.052 | 0.004 |
| MFL | **0.831** | 0.146 | -0.309 | 0.309 | -0.002 |
| LNL | **0.774** | 0.190 | -0.332 | 0.346 | -0.059 |
| LRL | **0.945** | -0.022 | 0.117 | 0.124 | -0.027 |
| LPL | **0.976** | -0.044 | 0.083 | 0.135 | -0.030 |
| AIOL | **0.964** | -0.021 | -0.012 | 0.139 | -0.012 |
| NL | **0.781** | -0.159 | 0.419 | -0.027 | 0.010 |
| SL | **0.952** | -0.145 | 0.141 | 0.080 | 0.033 |
| MPL | **0.945** | -0.140 | 0.150 | 0.002 | 0.017 |
| OPL | **0.945** | -0.086 | 0.126 | 0.034 | -0.024 |
| LIL | **0.828** | -0.068 | 0.289 | -0.001 | 0.023 |
| UPRL | -0.437 | 0.257 | 0.297 | 0.354 | 0.374 |
| LO1 | **0.725** | 0.143 | -0.343 | -0.052 | -0.308 |
| LO2 | **0.696** | 0.092 | -0.051 | -0.078 | -0.484 |

Table 5. continued.

| | | | | | |
|---|---|---|---|---|---|
| MB | **0.776** | 0.258 | -0.023 | -0.189 | 0.095 |
| OCB | 0.436 | **0.517** | 0.270 | -0.323 | 0.316 |
| POPB | **0.661** | 0.330 | -0.127 | -0.262 | 0.328 |
| FMB | 0.121 | **0.655** | 0.144 | -0.325 | -0.044 |
| FMH | -0.099 | **0.572** | 0.413 | 0.341 | -0.454 |
| LFB | 0.206 | 0.393 | -0.227 | 0.462 | 0.205 |
| ZB | **0.863** | 0.089 | -0.165 | -0.185 | -0.158 |
| LOB | **0.572** | 0.075 | -0.373 | 0.071 | 0.132 |
| OB | **0.840** | -0.001 | -0.238 | -0.229 | -0.106 |
| NB | **0.575** | 0.097 | -0.014 | -0.174 | 0.133 |
| IB | **0.675** | 0.095 | -0.153 | -0.325 | -0.155 |
| BNCH | **0.671** | 0.291 | 0.164 | 0.088 | -0.029 |
| Eigenvalue | 21.32 | 1.86 | 1.59 | 1.34 | 1.18 |
| Proportion | 62.71 | 5.46 | 4.69 | 3.95 | 3.46 |
| Cumulative | 62.71 | 68.17 | 72.85 | 76.81 | 80.27 |

**Bold: absolute > 0.5.**

Table 6. Principal components of the mandible which account for more than 1 of eigenvalue from PCA.

| | Mandible | |
|---|---|---|
| Variable | PC1 | PC2 |
| MLA | **0.789** | **0.581** |
| MLC | **0.783** | **0.559** |
| Gp2L | **0.671** | **0.650** |
| GDFL | **0.754** | **0.537** |
| LPRL | **-0.892** | 0.097 |
| DL | **0.863** | 0.331 |
| AVRH | **0.717** | **0.567** |
| MVRH | **0.650** | **0.625** |
| OVRH | **0.593** | **0.672** |
| MH1 | -0.033 | **0.891** |
| MH2 | 0.468 | **0.713** |
| Eigenvalue | 8.14 | 1.11 |
| Proportion | 74.01 | 10.06 |
| Cumulative | 74.01 | 84.06 |

**Bold: absolute > 0.5.**

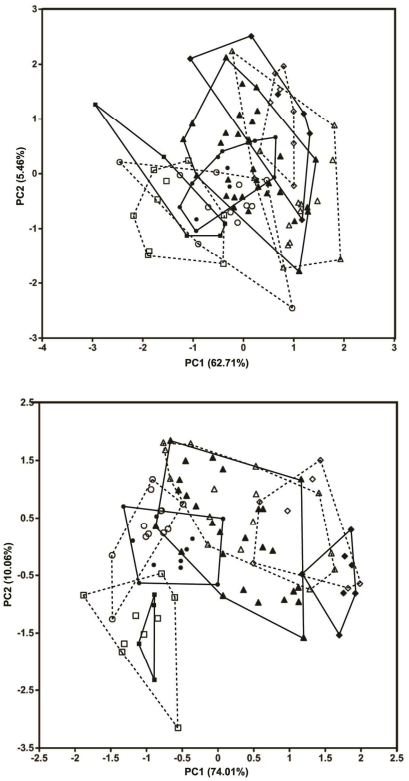


Fig. 3. Two-dimensional scatter plots of the first and second principal component scores of cranium (up) and mandible (down). ■□:age group 1, ●○: age group 2, ▲Δ: age group 3, ◆◇: age group 4, closed: male, opened: female.

*Allometric analysis*: There were significant differences of α in allometry equations between the sexes in 5 traits. The $\alpha$ value for CBL was larger in the female, while those for 4 traits (OCB, POPB, LOB and IB) were larger in the male (Fig. 4). Trends of allometry for OCB, POPB and LOB were different between sexes, while IB was positively allometric in both sexes. The allometric trends of common slopes between sexes were positively allometric for 20 traits (BL, PPL, BFL, VCL, NL, SL, MPL, OPL, LIL, NB, MLA, MLC, Gp2L, GDFL, DL, AVRH, MVRH, OVRH, MH1 and MH2), negatively allometric for 11 traits (NCL, MFL, LNL, AIOL, UPRL, MB, FMH, LFB, ZB, BNCH and LPRL) and isometric in 8 traits (SSL, BCL, LRL, LPL, LO1, LO2, FMB and OB) (Table 7). Among them, 4 variables (FMB, FMH, LFB and MH) showed no significant correlation with TL in either or both sexes. Therefore, estimation of $\alpha$ value for these variables may not be meaningful. Slope values for UPRL and LPRL were smaller than 0. Among the traits which showed no significant difference of slopes between sexes, significant difference of elevation between sexes was detected only in DL, but the difference was small.

Table 7. Results of the allometric analyses for each sex and comparisons of slopes and elevations between sexes.

| Variable | Male | | | | | Female | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *A* | CI | Trend | $r^2$ | | $\alpha$ | CI | Trend | $r^2$ | |
| CBL | 1.025 | 0.966 - 1.087 | I | 0.955 | *** | 1.112 | 1.051 - 1.176 | P | 0.968 | *** |
| BL | 1.097 | 1.021 - 1.178 | P | 0.935 | *** | 1.177 | 1.104 - 1.254 | P | 0.959 | *** |
| SSL | 0.974 | 0.869 - 1.093 | I | 0.833 | *** | 1.037 | 0.923 - 1.165 | I | 0.863 | *** |
| PPL | 1.456 | 1.306 - 1.623 | P | 0.851 | *** | 1.537 | 1.391 - 1.697 | P | 0.900 | *** |
| BCL | 1.207 | 0.998 - 1.461 | I | 0.535 | *** | 1.011 | 0.828 - 1.235 | I | 0.593 | *** |
| BFL | 1.140 | 1.072 - 1.212 | P | 0.953 | *** | 1.228 | 1.159 - 1.301 | P | 0.966 | *** |
| NCL | 0.779 | 0.660 - 0.920 | N | 0.645 | *** | 0.777 | 0.662 - 0.913 | N | 0.739 | *** |
| VCL | 1.457 | 1.311 - 1.619 | P | 0.859 | *** | 1.335 | 1.227 - 1.453 | P | 0.928 | *** |
| MFL | 0.874 | 0.740 - 1.033 | I | 0.644 | *** | 0.746 | 0.644 - 0.866 | N | 0.778 | *** |
| LNL | 0.822 | 0.682 - 0.991 | N | 0.553 | *** | 0.772 | 0.638 - 0.934 | N | 0.630 | *** |
| LRL | 0.975 | 0.875 - 1.086 | I | 0.851 | *** | 0.991 | 0.914 - 1.074 | I | 0.934 | *** |
| LPL | 0.991 | 0.946 - 1.039 | I | 0.972 | *** | 0.993 | 0.960 - 1.027 | I | 0.989 | *** |
| AIOL | 0.879 | 0.821 - 0.941 | N | 0.942 | *** | 0.870 | 0.822 - 0.921 | N | 0.967 | *** |
| NL | 1.880 | 1.549 - 2.281 | P | 0.520 | *** | 1.654 | 1.401 - 1.952 | P | 0.722 | *** |
| SL | 1.324 | 1.228 - 1.428 | P | 0.928 | *** | 1.357 | 1.265 - 1.456 | P | 0.950 | *** |
| MPL | 1.349 | 1.210 - 1.505 | P | 0.849 | *** | 1.337 | 1.241 - 1.441 | P | 0.944 | *** |
| OPL | 1.379 | 1.243 - 1.530 | P | 0.863 | *** | 1.359 | 1.255 - 1.471 | P | 0.937 | *** |
| LIL | 1.587 | 1.341 - 1.878 | P | 0.638 | *** | 1.462 | 1.249 - 1.712 | P | 0.749 | *** |

Table 7. continued.

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| UPRL | -1.178 | -1.539 - -0.902 | N | 0.076 | * | -1.059 | -1.384 - -0.811 | N | 0.265 | *** |
| LO1 | 0.947 | 0.761 - 1.179 | I | 0.383 | *** | 0.806 | 0.646 - 1.005 | I | 0.501 | *** |
| LO2 | 0.914 | 0.735 - 1.138 | I | 0.386 | *** | 0.898 | 0.716 - 1.127 | I | 0.473 | *** |
| MB | 0.890 | 0.733 - 1.080 | I | 0.519 | *** | 0.820 | 0.663 - 1.015 | I | 0.539 | *** |
| OCB | 1.113 | 0.861 - 1.439 | I | 0.146 | ** | 0.546 | 0.414 - 0.718 | N | 0.220 | ** |
| POPB | 0.978 | 0.780 - 1.226 | I | 0.342 | *** | 0.674 | 0.529 - 0.859 | N | 0.395 | *** |
| FMB | 1.357 | 1.041 - 1.767 | P | 0.094 | * | -0.943 | -1.282 - -0.694 | N | 0.022 | |
| FMH | -1.403 | -1.851 - -1.063 | N | 0.002 | | -1.084 | -1.477 - -0.795 | N | 0.005 | |
| LFB | 0.692 | 0.526 - 0.909 | N | 0.028 | | 0.639 | 0.472 - 0.865 | N | 0.051 | |
| ZB | 0.892 | 0.746 - 1.067 | I | 0.589 | *** | 0.829 | 0.700 - 0.983 | N | 0.708 | *** |
| LOB | 1.569 | 1.246 - 1.975 | P | 0.318 | *** | 1.074 | 0.824 - 1.401 | I | 0.275 | *** |
| OB | 0.975 | 0.811 - 1.173 | I | 0.564 | *** | 0.921 | 0.763 - 1.110 | I | 0.643 | *** |
| NB | 2.248 | 1.754 - 2.882 | P | 0.205 | *** | 1.713 | 1.332 - 2.204 | P | 0.350 | *** |
| IB | 2.233 | 1.895 - 2.632 | P | 0.656 | *** | 1.343 | 1.110 - 1.625 | P | 0.630 | *** |
| BNCH | 0.630 | 0.501 - 0.793 | N | 0.322 | *** | 0.619 | 0.492 - 0.778 | N | 0.464 | *** |
| MLA | 1.203 | 1.061 - 1.364 | P | 0.823 | *** | 1.166 | 1.069 - 1.272 | P | 0.926 | *** |
| MLC | 1.089 | 1.003 - 1.182 | P | 0.925 | *** | 1.107 | 1.046 - 1.173 | P | 0.968 | *** |
| Gp2L | 1.150 | 0.960 - 1.377 | I | 0.634 | *** | 1.108 | 0.975 - 1.259 | I | 0.840 | *** |
| GDFL | 1.212 | 1.030 - 1.425 | P | 0.705 | *** | 1.105 | 0.992 - 1.232 | I | 0.885 | *** |
| LPRL | -2.155 | -2.796 - -1.661 | N | 0.230 | *** | -1.827 | -2.326 - -1.435 | N | 0.418 | *** |
| DL | 1.994 | 1.713 - 2.322 | P | 0.741 | *** | 1.684 | 1.474 - 1.925 | P | 0.825 | *** |
| AVRH | 1.222 | 0.992 - 1.506 | I | 0.510 | *** | 1.382 | 1.181 - 1.617 | P | 0.757 | *** |

Table 7. continued.

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| MVRH | 1.299 | 1.041 - 1.621 | P | 0.447 | *** | 1.365 | 1.149 - 1.621 | P | 0.708 | *** |
| OVRH | 1.341 | 1.062 - 1.694 | P | 0.383 | *** | 1.406 | 1.191 - 1.659 | P | 0.728 | *** |
| MH1 | 1.754 | 1.312 - 2.343 | P | 0.041 | | 1.376 | 1.077 - 1.759 | P | 0.398 | *** |
| MH2 | 1.950 | 1.554 - 2.447 | P | 0.419 | *** | 1.518 | 1.252 - 1.840 | P | 0.633 | *** |

Table 7. continued.

| Variable | Slope comparison | | | | Intercept comparison | | | |
|---|---|---|---|---|---|---|---|---|
| | M vs. F | $\alpha_c$ | CI | Trend | M vs. F | $\beta_M$ | $\beta_F$ | $\beta_c$ |
| CBL | M < F * | - | | | | | | |
| BL | | 1.141 | 1.086 - 1.198 | P | | | | -0.371 |
| SSL | | 1.005 | 0.926 - 1.090 | I | | | | -0.263 |
| PPL | | 1.500 | 1.393 - 1.613 | P | | | | -1.611 |
| BCL | | 1.109 | 0.965 - 1.276 | I | | | | -0.902 |
| BFL | | 1.186 | 1.135 - 1.238 | P | | | | -0.587 |
| NCL | | 0.778 | 0.694 - 0.873 | N | | | | 0.237 |
| VCL | | 1.381 | 1.293 - 1.478 | P | | | | -1.162 |
| MFL | | 0.800 | 0.715 - 0.896 | N | | | | 0.192 |
| LNL | | 0.797 | 0.698 - 0.910 | N | | | | 0.146 |
| LRL | | 0.985 | 0.924 - 1.050 | I | | | | -0.063 |
| LPL | | 0.992 | 0.966 - 1.020 | I | | | | -0.004 |
| AIOL | | 0.874 | 0.837 - 0.913 | N | | | | 0.124 |
| NL | | 1.744 | 1.539 - 1.982 | P | | | | -2.159 |
| SL | | 1.342 | 1.275 - 1.412 | P | | | | -1.080 |
| MPL | | 1.341 | 1.261 - 1.426 | P | | | | -1.003 |
| OPL | | 1.366 | 1.284 - 1.455 | P | | | | -1.182 |
| LIL | | 1.519 | 1.355 - 1.704 | P | | | | -1.708 |
| UPRL | | -1.116 | -1.349 - -0.925 | N | | | | 3.858 |

Table 7. continued.

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| LO1 | | 0.873 | 0.748 - 1.022 | I | | | | -0.536 |
| LO2 | | 0.907 | 0.775 - 1.060 | I | | | | -0.616 |
| MB | | 0.857 | 0.744 - 0.989 | N | | | | -0.234 |
| OCB | M > F *** | - | | | | | | |
| POPB | M > F * | - | | | | | | |
| FMB | | 1.161 | 0.948 - 1.424 | I | | | | -1.424 |
| FMH | | -1.250 | -1.539 - -1.016 | N | | | | 3.945 |
| LFB | | 0.667 | 0.545 - 0.817 | N | | | | 0.096 |
| ZB | | 0.858 | 0.759 - 0.971 | N | | | | -0.052 |
| LOB | M > F * | - | | | | | | |
| OB | | 0.948 | 0.832 - 1.080 | I | | | | -0.258 |
| NB | | 1.964 | 1.644 - 2.353 | P | | | | -3.158 |
| IB | M > F *** | - | | | | | | |
| BNCH | | 0.625 | 0.532 - 0.734 | N | | | | 0.233 |
| MLA | | 1.178 | 1.097 - 1.265 | P | | | | -0.489 |
| MLC | | 1.101 | 1.051 - 1.154 | P | | | | -0.337 |
| Gp2L | | 1.122 | 1.012 - 1.244 | P | | | | -0.548 |
| GDFL | | 1.136 | 1.039 - 1.245 | P | | | | -0.506 |
| LPRL | | -1.971 | -2.355 - -1.652 | N | | | | 5.760 |
| DL | | 1.812 | 1.635 - 2.011 | P | M > F ** | -2.408 | -2.422 | |
| AVRH | | 1.323 | 1.165 - 1.498 | P | | | | -1.332 |
| MVRH | | 1.340 | 1.171 - 1.533 | P | | | | -1.392 |

Table 7. continued.

| | | | | |
|---|---|---|---|---|
| OVRH | 1.384 | 1.210 - 1.583 | P | -1.278 |
| MH1 | 1.521 | 1.261 - 1.840 | P | -2.221 |
| MH2 | 1.684 | 1.451 - 1.961 | P | -2.607 |

CI: 95% confidence intervals of α values. I: isometry, P: positive allometry, N: negative allometry, αc: common slope value when slope values of both sexes were not different, βM and βF: Intercept for male and female when the slope was not different between sexes. βc: Common intercept value when the intercept was not different between sexes, *: $0.01 < p < 0.05$, **: $0.001 < p < 0.01$, ***: $p < 0.001$.

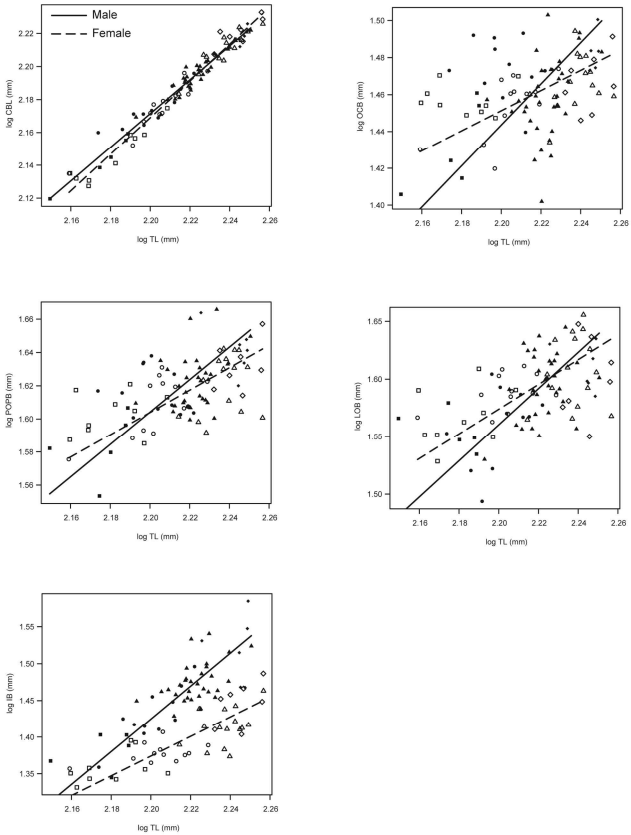


Fig. 4. Biplots of TL and 5 traits which showed significant difference of slopes between sexes in allometric analyses. ■□: age group 1, ●○:age group 2, ▲Δ: age group 3, ◆◇: age group 4, closed: male, opened: female.

## DISCUSSION

The present results show considerable growth variation between sexes in *H. i. argyropus*. Overall size variation of the male is expressed by PC1 in the cranium and the mandible, and the growth seems to be variable and continuous with highly overlapping scatter pattern among neighboring age groups. In the case of the cranium and the mandible of the female, the level of overlap between age groups 2 and 3 was not so high. On the other hand, the level of the overlap between age groups 3 and 4 was high.

A total of 5 variables were allometrically different in both sexes. Allometric difference of OCB, POPB and LOB between sexes was considered to relate to the difference of growth of the braincase apparatus, and it means that the growth of the braincase in the male is faster than that in the female. Allometric difference of CBL was related to growth of the skull size, and it shows that the size growth in the female is faster than in the male. Although both TL and CBL are representatives of skull size, the growth pattern of the two traits is shown to be slightly different, because of the characteristics of

traits. The size of TL might be affected by the size of FMH. However, CBL does not have such a characteristic. In this study, FMH does not show any differences among age groups in both sexes. However, the size of FMH slightly decreased from age group 1 to age group 4 (Table 3). In PCA, FMH shows a negative correlation with PC1, although the loading value is very low (Table 5). Similarly with PCA, the allometric test also shows negative allometric growth pattern of FMH (Table 7). Because the growth pattern of TL is affected by FMH, it is considered that the growth pattern of TL is slightly different from that of CBL. The outstanding characteristics in the skull growth are the decrease in the size of the premolar row (UPRL of cranium and LPRL of mandible) in age groups 1 to 4, and the evidence that the length of the upper and lower premolar rows is shorter than the upper and lower molar rows. This kind of growth pattern might be the result of evolutionary adaptation by the feeding type. Although some ecological studies of the Korean water deer have been done, the feeding style of this species has remained unclear, whether it is a browser or a grazer (Lee, 2003). Although Clauss et al. designated Chinese water deer as

an intermediate feeder based on the masseter mass analysis, both Korean and Chinese water deer have been recently classified as a browser based on their feeding habit and rumen structure (Hofmann et al., 1988; Kim et al., 2011). There is a different trend in the size of the premolar row between grazing and browsing ungulate species (Mendoza et al., 2002). Ruminant artiodactyls which have a browsing dietary habit usually show a relatively longer lower premolar row (premolars: 70% of the molar row) than that of grazing ruminants (premolars: 45% of the molar row) (Mendoza et al., 2002). According to the result of the present study, the proportion of the lower premolar row length to the lower molar row length was 73.6% in age group 3 and 64.0% in age group 4 in the male, and it was 71.8% and 64.0% in the female, respectively. This proportion is similar to the proportion of the browsing artiodactyl, and the feeding habit might cause the distinctive growth pattern for length of the lower premolar row. The size of molar row was more developed, and the size of premolar row was equivalently reduced. The proportion of the premolar row to the molar row of age groups 1 and 2 is not shown in this study, because the molars were not fully

erupted in these age groups.

The method which measures the breadth across the incisive bone of the canines was used in order to ensure the growth of the canines instead of measuring the size of the canines directly in this study. Canines of water deer are known to be mainly used for marking territory and used as the fighting weapon in male-male competition during the breeding season (Cooke and Farrell, 1998). Furthermore, water deer with broken canines were commonly observed in the wild (Cooke and Farrell, 1998). Because of these evidences, an indirect method to measure the size of the breadth across the incisive bone was used in this study.

Maximum longevity of the Korean water deer in the wild has not been reported yet, but the reported lifespan of the Chinese water deer is about 10–12 years (Allen, 1940; Nowak, 1991; Putman, 1988). Canines of the male water deer are one of the outstanding characteristics of this species, and it is known that canines are completely grown from age 18 to 24 months (Cooke and Farrell, 1998). It means that the specimens classified to age group 4 in our

study are at least over 18 months. In this study, age groups are divided into only 4 groups, which is surely a shortcoming of this research. For water deer, how to determine the actual age has still not been determined. Thus, it is difficult to compare the actual age with the age groups used in this study. With field observation and captured individual, the correct identification of actual age is needed for the further research on this species.

In conclusion, the growth pattern of the Korean water deer shows slight differences and similarities between the male and the female. Canines of the male were the most outstanding characteristic between the two sexes. Although differences between the sexes were found in some traits, those were not remarkable, except for some length dimensions in the cranium and mandible in age group 3.

## Part II. Sexual Dimorphism of Craniomandibular Size in the Korean Water Deer, *Hydropotes inermis argyropus*

## INTRODUCTION

Being the species that has retained the primitive morphology of deer, the water deer (*Hydropotes inermis*) has been cited as a critical species for the understanding of the evolutionary history of Cervidae (Sun and Dai, 1995). Taxonomically, genus *Hydropotes* is currently considered to include the Chinese subspecies (*H. i. inermis*) and the Korean subspecies (*H. i. argyropus*) (Geist, 1998). Several fundamental studies of the Chinese subspecies have been reported. For example, male-female association and mating system of the Chinese water deer were observed using 23 wild individuals (♂: 12 and ♀: 11) (Sun and Dai, 1995), and uniparental female care of the Chinese water deer was reported using introduced individuals in England (Zhang, 1998). In addition to this, intraspecific genetic

diversity of the nuclear and mitochondrial DNA in this species has been reported (Hu et al., 2006; Hu et al., 2007). Until recently, very little was known about the morphology of the Korean water deer, but a series of our studies have provided basic information on the skull growth pattern, the lamination of the masticatory muscle and the genital organ anatomy (Kim et al., 2013(a); Sasaki et al., 2013; Sohn and Kimura, 2012; Sohn et al., 2013). Here, I report for the first time the sexual dimorphism pattern of the Korean water deer.

Sexual dimorphism of size and shape is a fairy common phenomenon between conspecific males and females, and may arise from ecological differences in many animal taxa. In particular, sexual dimorphism of ungulate species has been described on the basis of various hypotheses, including sexual selection, food dispersion, social behavior, and mating system (Geist and Bayer, 1988; Ralls, 1976). Describing sexual dimorphism of the craniomandibular morphology in the Korean water deer can provide basic insights to understand the social system of this species.

Thus, I investigated the degree of sexual dimorphism in craniomandibular traits in the Korean water deer.

## MATERIALS AND METHODS

In this study, I examined sexual dimorphism using cranial measurements of 52 individuals (male: 31, female: 21) and mandibular measurements of 54 individuals of *H. i. argyropus* (male: 33, female: 21). Only adult specimens in which the upper and lower third molars (M3 and m3) are fully erupted, were used in order to avoid age related bias. I conducted univariate and multivariate comparisons by using 15 cranial and 11 mandibular measurements (Table 8, Fig. 5), based on my previous report with slight modification (Kim et al., 2013(a)). As univariate analysis, I compared the mean and variance values of each measurement by Welch's *t*-test and F-test, respectively. For multivariate analysis, I conducted principal component analysis (PCA) based on correlation matrix using natural log-transformed values of cranial and mandibular measurements (cPCA and mPCA) and discriminant analysis (DA) using the scores from all PC axes of cranial and mandibular measurements (cDA and mDA), separately. PCA was

conducted with PASW Statistics v18 program (IBM Acquires SPSS Inc., Chicago, IL, U.S.A.) and DA was conducted with the software PAST version 2.07 (Hammer et al., 2001).

I performed allometric test to investigate differences in skull proportion using total length (TL) and mandible length from the angle (MLA) as independent variables of crania and mandible, respectively. Allometric analysis is usually employed to describe differences of shape between sexes (Warton and Ormerod, 2005). I used bivariate allometry equation, and the equation is expressed as the formula $y = b \cdot x^{\alpha}$ ($\beta = log\ b$). In this formula, $\alpha$-value is allometry coefficient (equilibrium constant), and $\beta$-value is the initial growth index. The common logarithmic conversion of that formula is $log\ y = \alpha \cdot log\ x + \beta$ (Warton and Ormerod, 2005). I compared slope ($\alpha$) and intercept ($\beta$) of allometry equations between sexes using two different independent variables; TL for cranium and MLA for mandible. Reduced major axis (RMA) was used to estimate the allometry coefficient (Warton and Ormerod, 2005).

Testing whether the slopes ($\alpha$) is significantly different from 1.0 and the comparison of the slopes between the male and the female were conducted by using SMATR package (Warton and Ormerod, 2005).

Table 8. List of cranial and mandibular measurements of the Korean water deer used in this study.

| Category | Acronym | Measurement |
|---|---|---|
| CRANIUM | TL | Total length |
| | CBL | Condylobasal length |
| | BL | Basal length |
| | NCL | Neurocranium length |
| | VCL | Viscerocranium length |
| | NL | Greatest length of the nasals |
| | UCRL | Length of the upper cheektooth row |
| | OCB | Greatest breadth of the occipital condyles |
| | LFB | Least frontal breadth |
| | ZB | Zygomatic breadth |
| | LOB | Least breadth between the orbits |
| | OB | Greatest breadth across the orbits |
| | NB | Greatest breadth across the nasals |
| | IB | Greatest breadth across the incisive bone |
| | BNCH | Basion - the highest point of the superior nuchal crest |
| MANDIBLE | MLA | Mandible length from the angle |
| | GM3L | Gonion - aboral border of the alveolus of M3 |
| | HRL | Length of the horizontal ramus |
| | Gp2L | Gonioncaudale – oral border of the alveleolus of p2 |
| | LCRL | Length of the lower cheektooth row |
| | LMRL | Length of the lower molar row |
| | LPRL | Length of the lower premolar row |

Table 8. continued.

| | |
|---|---|
| DL | Length of the diastema |
| AVRH | Aboral height of the vertical ramus |
| MVRH | Middle height of the vertical ramus |
| OVRH | Oral height of the vertical ramus |

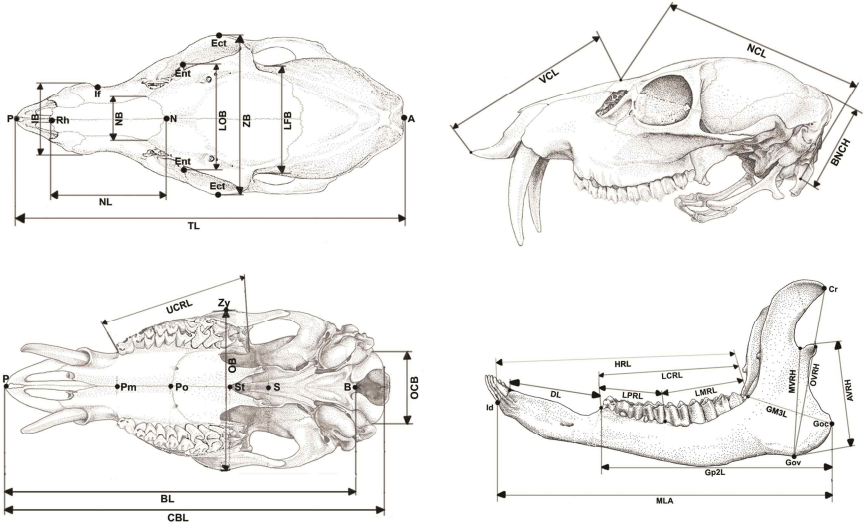


Fig. 5. Skull measurements of the Korean water deer used in this study.

## RESULTS

*Univariate analyses*: Mean values were different between sexes in 7 variables of cranium (TL, CBL, BL, NCL, VCL, ZB and IB) and 3 variables of mandible (MLA, 9 GM3L and Gp2L) (Table 9). Among these 10 traits, 9 variables were larger in female specimens, and the ratio of both sexes ranged from 95.99% in VCL to 97.83% in ZB, while IB was larger in males (112.42%). Differences of variance were not observed between sexes.

Table 9. Descriptive statistics and results of comparison of cranial and mandubular variables in each sex of the Korean water deer.

| Variable | Sex | N | Mean | Ratio (%) | Welch's *t*-test | | Variance | Ratio (%) | *F*-test | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | *t* | *p* | | | *F* | *p* |
| TL | male | 31 | 168.16 | 96.84 | -4.476 | **0.001** | 19.05 | 101.76 | 1.018 | 0.988 |
| | female | 21 | 173.65 | | | | 18.72 | | | |
| CBL | male | 31 | 157.96 | 96.64 | -4.457 | **0.001** | 21.01 | 118.83 | 1.188 | 0.698 |
| | female | 21 | 163.45 | | | | 17.68 | | | |
| BL | male | 31 | 147.47 | 96.20 | -4.869 | **0.001** | 19.17 | 112.17 | 1.122 | 0.802 |
| | female | 21 | 153.29 | | | | 17.09 | | | |
| NCL | male | 31 | 92.86 | 97.83 | -2.370 | **0.024** | 6.20 | 53.73 | 1.862 | 0.120 |
| | female | 21 | 94.92 | | | | 11.54 | | | |
| VCL | male | 31 | 81.36 | 95.99 | -3.661 | **0.001** | 13.74 | 155.78 | 1.556 | 0.305 |
| | female | 21 | 84.76 | | | | 8.82 | | | |
| NL | male | 31 | 53.01 | 97.16 | -1.508 | 0.139 | 12.03 | 85.87 | 1.165 | 0.690 |
| | female | 21 | 54.56 | | | | 14.01 | | | |
| UCRL | male | 31 | 50.02 | 101.21 | 0.886 | 0.381 | 4.89 | 77.74 | 1.285 | 0.522 |
| | female | 21 | 49.42 | | | | 6.29 | | | |
| OCB | male | 31 | 28.86 | 98.63 | -1.150 | 0.256 | 2.28 | 221.65 | 2.215 | 0.067 |
| | female | 21 | 29.26 | | | | 1.03 | | | |

Table 9. continued.

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| LFB | male | 31 | 37.97 | 100.24 | 0.207 | 0.837 | 1.55 | 46.69 | 2.137 | 0.058 |
| | female | 21 | 37.88 | | | | 3.32 | | | |
| ZB | male | 31 | 71.94 | 97.66 | -2.168 | **0.036** | 6.48 | 73.30 | 1.363 | 0.433 |
| | female | 21 | 73.66 | | | | 8.84 | | | |
| LOB | male | 31 | 39.62 | 97.92 | -1.124 | 0.269 | 4.40 | 50.06 | 1.996 | 0.084 |
| | female | 21 | 40.46 | | | | 8.79 | | | |
| OB | male | 31 | 71.32 | 98.86 | -1.063 | 0.294 | 6.53 | 81.52 | 1.227 | 0.599 |
| | female | 21 | 72.14 | | | | 8.01 | | | |
| NB | male | 31 | 16.51 | 100.49 | 0.167 | 0.868 | 2.95 | 92.77 | 1.078 | 0.834 |
| | female | 21 | 16.43 | | | | 3.18 | | | |
| IB | male | 31 | 29.97 | 112.42 | 5.315 | **0.001** | 6.61 | 180.11 | 1.801 | 0.173 |
| | female | 21 | 26.66 | | | | 3.67 | | | |
| BNCH | male | 31 | 41.68 | 98.49 | -1.621 | 0.112 | 1.99 | 104.74 | 1.044 | 0.939 |
| | female | 21 | 42.32 | | | | 1.90 | | | |
| MLA | male | 33 | 137.49 | 97.95 | 2.361 | **0.022** | 22.30 | 131.95 | 1.319 | 0.521 |
| | female | 21 | 140.37 | | | | 16.90 | | | |
| GM3L | male | 33 | 34.03 | 93.44 | 2.411 | **0.020** | 11.97 | 91.94 | 1.087 | 0.812 |
| | female | 21 | 36.42 | | | | 13.02 | | | |
| HRL | male | 33 | 104.46 | 99.96 | 0.046 | 0.964 | 6.55 | 66.77 | 1.496 | 0.301 |
| | female | 21 | 104.50 | | | | 9.81 | | | |
| Gp2L | male | 33 | 89.48 | 97.26 | 3.643 | **0.001** | 7.45 | 140.83 | 1.407 | 0.426 |
| | female | 21 | 92.00 | | | | 5.29 | | | |

Table 9. continued.

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| LCRL | male | 33 | 56.62 | 100.18 | -0.110 | 0.913 | 8.12 | 72.44 | 1.381 | 0.405 |
| | female | 21 | 56.52 | | | | 11.21 | | | |
| LMRL | male | 33 | 33.11 | 98.69 | 1.245 | 0.219 | 1.91 | 138.41 | 1.381 | 0.453 |
| | female | 21 | 33.55 | | | | 1.38 | | | |
| LPRL | male | 33 | 23.89 | 102.36 | -0.682 | 0.499 | 6.75 | 73.29 | 1.364 | 0.423 |
| | female | 21 | 23.34 | | | | 9.21 | | | |
| DL | male | 33 | 42.55 | 99.56 | 0.231 | 0.818 | 11.23 | 148.74 | 1.487 | 0.355 |
| | female | 21 | 42.74 | | | | 7.55 | | | |
| AVRH | male | 33 | 41.22 | 97.56 | 1.621 | 0.114 | 3.46 | 54.66 | 1.831 | 0.123 |
| | female | 21 | 42.25 | | | | 6.33 | | | |
| MVRH | male | 33 | 39.17 | 97.73 | 1.392 | 0.173 | 3.86 | 60.41 | 1.656 | 0.198 |
| | female | 21 | 40.08 | | | | 6.39 | | | |
| OVRH | male | 33 | 63.74 | 97.55 | 1.479 | 0.148 | 11.26 | 64.94 | 1.539 | 0.269 |
| | female | 21 | 65.34 | | | | 17.34 | | | |

Ratio: ratios of mean and variance, male/female × 100 (%). **Bold**: $p < 0.05$

*PCA*: In the cPCA, the first 4 components which account for more than 1 value of eigenvalue explained 39.85, 16.06, 10.57 and 7.60% of the total variation, respectively (Table 10). Factor loadings for cPC1 were large in TL (0.793), CBL (0.793), BL (0.797), VCL (0.949) and NL (0.842). Factor loadings for cPC2 were large in NCL (0.894), OCB (0.640), ZB (0.631), LOB (0.678), OB (0.630) and BNCH (0.542); those for cPC3 were large in UCRL (0.718), LFB (0.569) and ZB (-0.532). For the cPC4 that explains 7.60% of the total variation, the loading values of NB (0.647) and IB (0.810) were large.

In the mPCA, the first 3 components which account for more than 1 value of eigenvalue explained 50.50, 24.42 and 9.15% of the total variation, respectively (Table 11). Factor loadings of mPC1 were large and correlated positively in MLA (0.884), GM3L (0.887), Gp2L (0.701) and DL (0.788). On the other hand, LCRL (-0.646) and LPRL (-0.730) were negatively correlated with mPC1. Factor loadings of mPC2 were large and positively correlated with 3 traits

of the height of the vertical ramus, AVRH (0.895), MVRH (0.937) and OVRH (0.849). Those of mPC3 were positively correlated with HRL (0.748) and 3 traits of teeth, LCRL (0.724), LMRL (0.843) and LPRL (0.516).

In the plots of cPCA and mPCA, samples of both sexes overlapped each other. However, the degree of overlapping was slightly different. Factor loading values of cPC1 and cPC2 between two sexes were significantly different ($p = 0.003$ for cPC1 and $p = 0.029$ for cPC2). On the other hand, factor loading values of mPCA between the male and the female were not significantly different ($p = 0.056$ for mPC1 and $p = 0.428$ for mPC2) (Fig. 6).

Table 10. Principal components of cranium which account for more than 1 of eigenvalue from cPCA

| | Cranium | | | |
|---|---|---|---|---|
| Variable | PC1 | PC2 | PC3 | PC4 |
| TL | **0.793** | 0.488 | -0.213 | -0.079 |
| CBL | **0.793** | 0.438 | -0.275 | -0.132 |
| BL | **0.797** | 0.451 | -0.305 | -0.123 |
| NCL | 0.207 | **0.894** | 0.010 | -0.088 |
| VCL | **0.949** | 0.030 | -0.088 | -0.064 |
| NL | **0.842** | -0.160 | 0.172 | 0.157 |
| UCRL | -0.033 | -0.052 | **0.718** | -0.217 |
| OCB | 0.285 | **0.640** | 0.284 | 0.103 |
| LFB | -0.091 | 0.235 | **0.569** | 0.166 |
| ZB | 0.281 | **0.631** | **-0.532** | 0.210 |
| LOB | -0.101 | **0.678** | 0.023 | 0.289 |
| OB | 0.073 | **0.630** | -0.491 | 0.367 |
| NB | 0.485 | 0.152 | -0.017 | **0.647** |
| IB | -0.267 | 0.151 | -0.109 | **0.810** |
| BNCH | 0.495 | **0.542** | 0.263 | 0.174 |
| Eigenvalue | 5.98 | 2.41 | 1.59 | 1.14 |
| Proportion | 39.85 | 16.06 | 10.57 | 7.60 |
| Cumulative | 39.85 | 55.91 | 66.48 | 74.08 |

**Bold: absolute > 0.5.**

Table 11. Principal components of the mandible which account for more than 1 of eigenvalue from mPCA

| | Mandible | | |
|---|---|---|---|
| Variable | PC1 | PC2 | PC3 |
| MLA | **0.884** | 0.367 | 0.225 |
| GM3L | **0.887** | 0.321 | -0.130 |
| HRL | 0.002 | 0.312 | **0.748** |
| Gp2L | **0.701** | 0.299 | 0.423 |
| LCRL | **-0.646** | -0.113 | **0.724** |
| LMRL | 0.071 | 0.045 | **0.843** |
| LPRL | **-0.730** | -0.165 | **0.516** |
| DL | **0.788** | 0.278 | -0.093 |
| AVRH | 0.353 | **0.895** | 0.027 |
| MVRH | 0.260 | **0.937** | 0.069 |
| OVRH | 0.312 | **0.849** | 0.235 |
| Eigenvalue | 5.56 | 2.69 | 1.01 |
| Proportion | 50.50 | 24.42 | 9.15 |
| Cumulative | 50.50 | 74.92 | 84.07 |

**Bold: absolute > 0.5.**

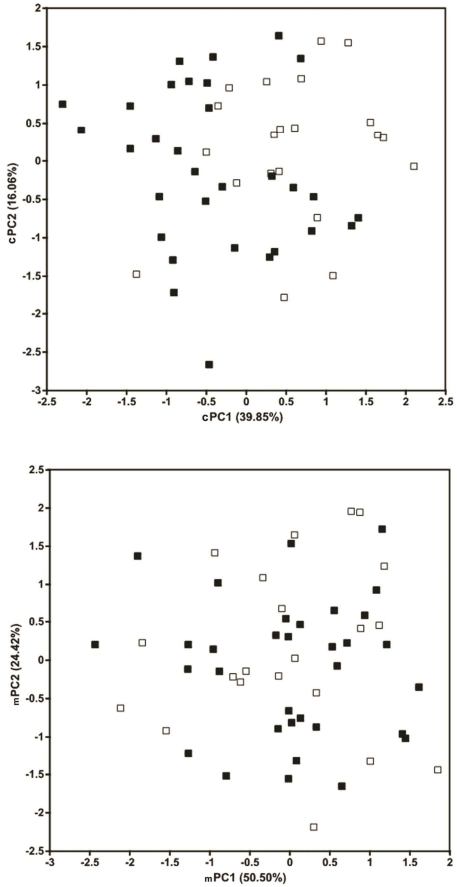


Fig. 6. Two-dimensional plots of the first and second principal component axes in cranium (up) and mandible (down) measurements. Closed: male and open: female.

*DA*: Using the scores from all PC axes, 96.15 and 76.92% of specimens were correctly classified into each sex, respectively (Fig. 7). Standardized canonical discriminant coefficients for cDA were large in BL (1.615), VCL (-0.980) and BL (-1.188) in cDA and large in MLA (2.649), DL (-1.961), AVRH (0.938) and MVRH (-0.903) in mDA. The result of cDA discriminated between the sexes significantly ($p < 0.05$), however, the result of mDA did not discriminate both the sexes significantly ($p > 0.05$).

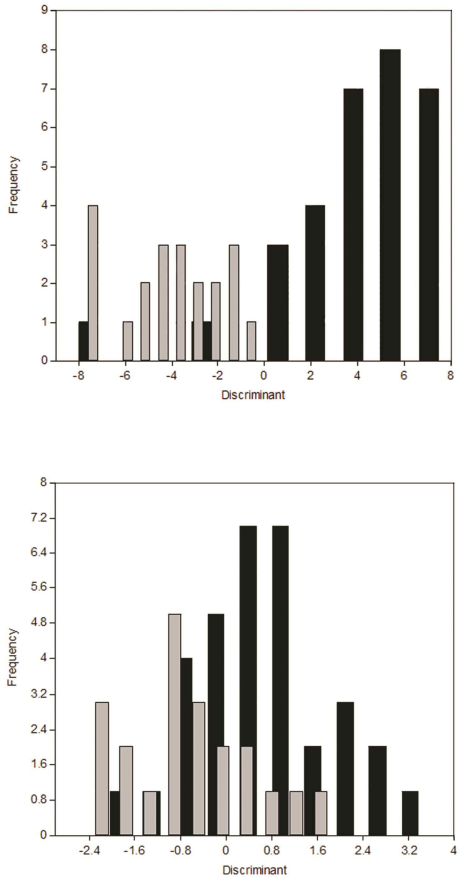


Fig. 7. Frequency distribution of DA1 scores by cDA (up) and mDA (down). Black: male, grey: female.

*Allometry*: Allometric comparisons between both sexes of this species showed that there were no significant differences in the slope of 14 cranial and 10 mandibular measurements (Table 12).

Table 12. Results of allometric analyses for each sex and comparisons of slopes and elevations between sexes.

| Vari-able | Male | | | | | | | Female | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | n | *A* | CI | Trend | $r^2$ | | $\beta$ | n | $\alpha$ | CI | Trend | $r^2$ | | $\beta$ |
| CBL | 31 | 1.124 | 0.919 - 1.375 | I | 0.715 | *** | -0.700 | 21 | 1.038 | 0.846 - 1.275 | I | 0.815 | *** | -0.258 |
| BL | 31 | 1.144 | 0.987 - 1.326 | I | 0.847 | *** | -0.870 | 21 | 1.081 | 0.875 - 1.336 | I | 0.803 | *** | -0.542 |
| NCL | 31 | 1.045 | 0.758 - 1.441 | I | 0.258 | ** | -0.829 | 21 | 1.417 | 0.971 - 2.067 | I | 0.351 | ** | -2.752 |
| VCL | 31 | 1.749 | 1.416 - 2.159 | P | 0.687 | *** | -4.563 | 21 | 1.400 | 0.988 - 1.983 | I | 0.453 | *** | -2.780 |
| NL | 31 | 2.559 | 1.893 - 3.460 | P | 0.351 | *** | -9.147 | 21 | 2.756 | 1.778 - 4.271 | P | 0.112 | | -10.21 |
| UCRL | 31 | -1.743 | -2.515 - -1.208 | N | 0.026 | | 12.84 | 21 | -2.027 | -3.208 - -1.281 | N | 0.020 | | 14.35 |
| OCB | 31 | 2.049 | 1.480 - 2.836 | P | 0.241 | ** | -7.137 | 21 | 1.387 | 0.893 - 2.154 | I | 0.105 | | -3.778 |
| LFB | 31 | -1.263 | -1.830 - -0.872 | N | 0.002 | | 10.11 | 21 | 1.922 | 1.210 - 3.052 | P | 0.004 | | -6.277 |
| ZB | 31 | 1.372 | 0.996 - 1.891 | P | 0.264 | ** | -2.758 | 21 | 1.618 | 1.132 - 2.314 | P | 0.421 | *** | -4.046 |
| LOB | 31 | 2.079 | 1.449 - 2.985 | P | 0.055 | | -6.978 | 21 | 2.944 | 1.858 - 4.664 | P | 0.015 | | -11.48 |
| OB | 31 | 1.405 | 1.005 - 1.964 | P | 0.191 | * | -2.932 | 21 | 1.583 | 1.022 - 2.450 | P | 0.117 | | -3.883 |
| NB | 31 | 4.142 | 2.997 - 5.725 | P | 0.248 | ** | -18.40 | 21 | 4.305 | 2.837 - 6.534 | P | 0.200 | * | -19.40 |
| IB | 31 | 3.383 | 2.339 - 4.893 | P | 0.011 | | -13.94 | 21 | 2.862 | 1.929 - 4.245 | P | 0.290 | * | -11.48 |

Table 12. continued.

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BNCH | 31 | 1.313 | 0.982 - 1.755 | I | 0.399 | *** | -2.999 | 21 | 1.311 | 0.836 - 2.055 | I | 0.061 | | -3.014 |
| GM3L | 33 | 2.892 | 2.371 - 3.527 | P | 0.702 | *** | -10.71 | 21 | 3.348 | 2.743 - 4.307 | P | 0.776 | *** | -13.40 |
| HRL | 33 | 0.720 | 0.536 - 0.967 | N | 0.332 | *** | 1.106 | 21 | 1.043 | 0.656 - 1.657 | I | 0.000 | | -0.506 |
| Gp2L | 33 | 0.891 | 0.715 - 1.110 | I | 0.663 | *** | 0.108 | 21 | 0.857 | 0.655 - 1.123 | I | 0.677 | *** | 0.283 |
| LCRL | 33 | -1.489 | -2.065 - -1.074 | N | 0.175 | * | 11.37 | 21 | -2.024 | -3.010 - -1.361 | N | 0.280 | * | 14.04 |
| LMRL | 33 | 1.221 | 0.861 - 1.731 | I | 0.053 | | -2.510 | 21 | -1.194 | -1.897 - -0.751 | N | 0.000 | | 9.413 |
| LPRL | 33 | -3.132 | -4.290 - -2.286 | N | 0.237 | ** | 18.58 | 21 | -4.376 | -6.254 - -3.062 | N | 0.423 | ** | 24.78 |
| DL | 33 | 2.330 | 1.914 - 2.837 | P | 0.707 | *** | -7.723 | 21 | 2.176 | 1.697 - 2.789 | P | 0.727 | *** | -7.002 |
| AVRH | 33 | 1.310 | 0.969 - 1.771 | I | 0.303 | *** | -2.731 | 21 | 2.025 | 1.456 - 2.815 | P | 0.512 | *** | -6.288 |
| MVRH | 33 | 1.462 | 1.074 - 1.990 | P | 0.269 | ** | -3.532 | 21 | 2.146 | 1.491 - 3.089 | P | 0.399 | ** | -6.921 |
| OVRH | 33 | 1.532 | 1.146 - 2.047 | P | 0.356 | *** | -3.388 | 21 | 2.124 | 1.513 - 2.982 | P | 0.481 | *** | -6.324 |

Table 12. continued.

| Variable | Slope comparison | | | |
|---|---|---|---|---|
| | M vs. F | $\alpha_c$ | CI | Trend |
| CBL | | 1.080 | 0.938 - 1.247 | I |
| BL | | 1.122 | 0.996 - 1.265 | I |
| NCL | | 1.192 | 0.926 - 1.522 | I |
| VCL | | 1.645 | 1.371 - 1.974 | P |
| NL | | 2.623 | 2.049 - 3.356 | P |
| UCRL | | -1.852 | -1.390 - -2.460 | N |
| OCB | | 1.779 | 1.367 - 2.328 | P |
| LFB | | -1.495 | -1.996 - -1.110 | N |
| ZB | | 1.481 | 1.164 - 1.874 | P |
| LOB | | 2.383 | 1.784 - 3.164 | P |
| OB | | 1.470 | 1.128 - 1.913 | P |
| NB | | 4.205 | 3.263 - 5.414 | P |
| IB | | 3.119 | 2.393 - 4.092 | P |
| BNCH | | 1.312 | 1.030 - 1.672 | P |

Table 12. continued.

| | | | |
|---|---|---|---|
| GM3L | 3.218 | 2.680 - 3.628 | P |
| HRL | 0.803 | 0.623 - 1.032 | I |
| Gp2L | 0.877 | 0.741 - 1.037 | I |
| LCRL | -1.693 | -1.306 - -2.176 | N |
| LMRL | 1.210 | 0.918 - 1.598 | I |
| LPRL | -3.643 | -2.844 - -4.611 | N |
| DL | 2.266 | 1.947 - 2.642 | P |
| AVRH | 1.605 | 1.257 - 2.015 | P |
| MVRH | 1.724 | 1.345 - 2.186 | P |
| OVRH | 1.766 | 1.401 - 2.204 | P |

$\alpha$: slope value, $\beta$: Intercept, CI: 95% confidence intervals of $\alpha$ values. I: isometry, P: positive allometry, N: negative allometry, $\alpha_c$: common slope value when slope values of both sexes were not different, *: $0.01 < p < 0.05$, **: $0.001 < p < 0.01$, ***: $p < 0.001$

## DISCUSSION

In the present study, I found that cranial traits are sexually dimorphic in the Korean water deer. In contrast, mandibular traits are not significantly different between the two sexes.

A total of 7 measurements of cranial traits (TL, CBL, BL, NCL, VCL, ZB and IB) and 3 measurements of mandibular traits (MLA, GM3L and Gp2L) were significantly different between sexes (Table 9). Except for IB, 9 measurements were larger in females than those in males. This is unique, given that males are generally larger than females in most mammalian taxa (Abouheif and Fairbairn, 1997; Loison et al., 1999; Pérez-Barbería and Gordon, 2000; Weckerly, 1998). There is a possible explanation for this unique sexual dimorphism of the water deer. The type of parental care could be an important factor for the sexual dimorphism (Ralls, 1976). Where only the female rears the offspring, the size of the female tends to be larger due to the energy consumed (e.g., in food foraging, suckling and combating against predators) than that of the male. Furthermore,

the selection among females favors a larger dam in species where offspring are raised without their sire, and selected big mothers are able to provide offspring with better conditions to make their survival predominance (Ralls, 1976). It has been reported that male water deer rarely participate in raising offspring (Zhang, 1998). Thus, the morphological differences, which were highlighted in this study, may be caused by the ecological characteristics in the female parental care, such as the additional energy consumption for raising offspring. Thus, I suggest that factors like the unique parental care influence the sexual dimorphism in this species.

Result from DA using cranial traits suggests that two sexes are well discriminated. Male Korean water deer exhibit characteristic canines, and I previously reported that IB is the representative trait reflecting the canines (Kim et al., 2013(a)). To test whether IB influenced the results of DA, the data from the cranial traits except IB were additionally conducted, and this result was identical to that of including IB ($p < 0.05$). The impact of the canines was not the sole

major factor that influenced the result of sexual dimorphism analysis. Therefore, I conclude that the sexual dimorphism (SD) of the water deer is mainly caused by size factors (TL, CBL, BL, NCL and VCL).

In summary, the sexual dimorphism of the Korean water deer was detected in cranial traits; however, it was not obvious in the mandibular traits. The skull size of the female was larger than that of male, and it has been suggested that this phenomenon was a consequence of an evolutionary factor like a type of the parental care.

# Part III. Cranial Morphological Difference in Two Subspecies of Water Deer in China and Korea

## INTRODUCTION

The water deer is the only species in the genus *Hydropotes*, subfamily Hydropotinae, family Cervidae. Two subspecies of water deer have traditionally been recognized. One is the Chinese water deer (*Hydropotes inermis inermis*, Swinhoe 1870), distributed in the lower Yangtze Basin, west to Hupeh in China. The other is the Korean water deer (*Hydropotes inermis argyropus*, Heude 1884), distributed throughout the whole of the Korean Peninsula (Allen, 1940). The subspecies classification has been based solely on the pelage color differences between the two populations. The Korean subspecies is reported to have darker pelage, with more reddish coloring in the head region compared to the Chinese subspecies (Tate, 1940). Otherwise, the two subspecies are very similar (Tate, 1940).

A recent molecular study (Koh et al., 2009) has raised questions about this subspecies classification. The authors studied the mitochondrial DNA (mtDNA) control region (927 bp) and cytochrome *b* gene (1,140 bp) sequences of both populuations. A total of 30 samples from three sites in China and 45 samples from five sites in Korea were used. The authors demonstrated two sympatric mtDNA clades (a major clade from China and Korea and a minor clade from Korea) with an average genetic distance of 2.1% in the control region and 1.3% in the cytochrome *b* gene, respectively. A total of 35 haplotypes from the control region were detected with more than 50% bootstrap values; a major clade consisted of 27 haplotypes from China and Korea and a minor clade had 8 haplotypes from Korea. Based on the cytochrome *b* gene, 25 haplotypes were identified. A major clade had 17 haplotypes from China and Korea, and a minor clade had 8 haplotypes from Korea. From this finding, the authors concluded that the current subspecific classification based on pelage color cannot be supported and pointed out the need to morphologically reexamine the validity of the

conventional subspecies classification.

In many cases, morphological variation related to adaptations to local climate is found between "subspecies" (i.e., Bergmann's rule; Bergmann, 1847). Bergmann's rule predicts that the average body size of a population in colder areas is generally larger than that in warmer regions due to physiological adaptations to colder environments. Numerous studies have tested Bergmann's rule, and the results have been equivocal, with some observations being consistent and others being inconsistent with the rule. According to Meiri and Dayan (2003), 97 of 149 mammal species from 12 orders (65.1%) follow Bergmann's rule. They reported that the validity of Bergmann's rule differed depending on the taxon. For example, Artiodactyla (seven species), Carnivora (43 species), Cetacea (one species), Chiroptera (13 species), Didelphimorphia (one species), Diprotodontia (six species), Hyracoidea (one species), Insectivora (10 species), Primates (six species) and Proboscidea (one species) generally comply with the rule, whereas Rodentia (51 species) does

not. Of the orders that do, some include fewer than 10 species or even only one species, which can be problematic for statistical analysis. That study included the order Artiodactyla, which includes the genus *Hydropotes*. The ranges of the two subspecies of water deer are at notably different latitudes (Chinese population: 30ºN, Korean population: 35–38ºN; Fig. 8), and the average lowest temperature differs considerably (about 2–8ºC in January in the Zhoushan archipelago, and about -10ºC in January in Korea). If Bergmann's rule holds, I would expect to find larger individuals in the Korean population.

Here I report the first detailed morphological study of water deer. I examined geographical variation in the skull using 36 measurements, and tested the validity of the conventional classification. The difference in sexual dimorphic patterns between the two populations was also examined. Based on the results, I suggest the need to reconsider the subspecies classification of water deer.

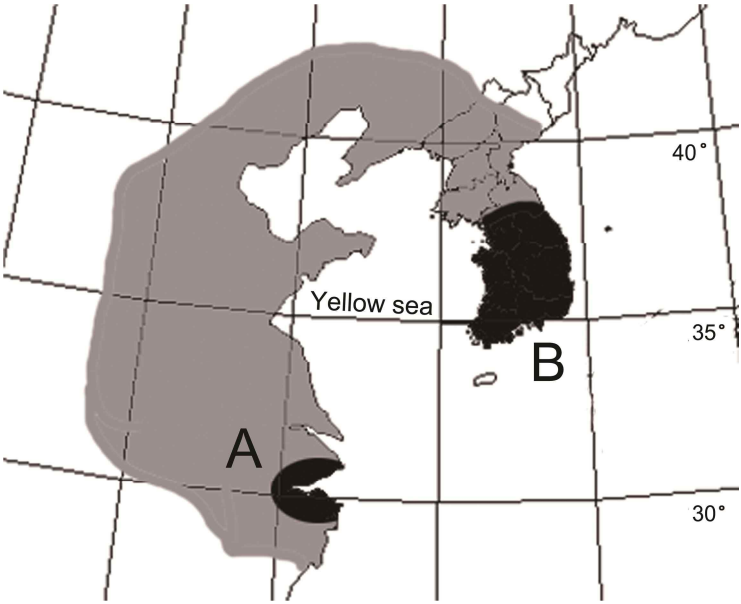


Fig. 8. Range map of water deer. Gray: original range map, black: distribution map of individuals used in this research. A: Chinese population, B: Korean population (redrawn from Whitehead, 1993).

# MATERIALS AND METHODS

## Sample collection

In total, 95 crania were examined: 50 *H. i. inermis* (♂=30, ♀=20) and 45 *H. i. argyropus* (♂=28, ♀=17) (Table 13). The specimens were from museums including East China Normal University (ECNU), Shanghai, China; the Shanghai Science and Technology Museum (SSTM), China; and the Chinese Academy of Sciences (CAS), Beijing, China. Although locality information of some of the Chinese specimens was missing, I considered all of these specimens as one Chinese population based on curator's testimony, and all specimens were from the habitat of the Chinese water deer, The Zhoushan Islands, where are located around 30°N latitude. For the Korean water deer, they are distributed around 35–38°N latitude and all specimens were collected by the Conservation Genome Resource Bank for Korean Wildlife (CGRB) and kept in the Laboratory of Anatomy and Cell Biology, College of Veterinary Medicine, Seoul National University. Specimens used were limited to adults with

fully erupted teeth to avoid age-related bias.

**Measurements and statistical analyses**

Following the definitions of von den Driesch (1976), 36 linear measurements (Fig. 9 and Table 14) were taken on the right side of each skull to the nearest 0.01 mm with digital vernier calipers (Mitutoyo, Tokyo, Japan).

As sexual dimorphism and geographical differences have not yet been reported in this species, I examined the differences in each skull measurement between males and females of both subspecies with a Student's *t*-test using PASW Statistics v18 program (IBM, Chicago, IL). All data were log-transformed before following multivariate analyses. Factor analysis (FA) and subsequent VARIMAX rotation were attempted to analyze the variation pattern. Standardized Cronbach's alpha value was estimated to assess the reliability of the factor reduction analysis. The statistical certainty of

assignment for individuals into their reference populations was evaluated by principal component analysis (PCA) and discriminant analysis (DA). These analyses were conducted using PAST version 2.12 (Hammer *et al*., 2001) for PCA, and XLSTAT version 7.5.2 (Addinsoft, New York, NY) for DA. Results of PCA were applied to the "75% rule" that defines the criteria for subspecies classification (Amadon, 1949).

The overall morphological similarity between and within the two populations were calculated using a Euclidean distance matrix by PopTools (Hood, 2010). Each Euclidean morphological distance value (*Ed*) was recalculated with the formula 1/(1+*Ed*) to set maximum and minimum values. Using this formula, all morphological distance values were converted into the range 0–1. Here, the pairwise similarity value approaches 1 with increasing morphological similarity between the two populations. The hierarchical cluster diagram was drawn using measurement data in PAST version 2.12 (Hammer *et al*., 2001). In this clustering analysis,

the neighbor-joining (NJ) clustering and the unweighted pair group method with arithmetic mean (UPGMA) clustering methods were conducted to test hierarchical topology among these specimens, and were assessed by 1,000 bootstrap replicates.

Table 13. The number and property of specimen in this study.

| | *H. i. argyropus* | *H. i. inermis* | Total |
|---|---|---|---|
| Male | 28 (Seoul National Univ.) | 30 (ECNU: 3, SSTM: 6 and CAS: 21) | 58 |
| Female | 17 (Seoul National Univ.) | 20 (SSTM: 2, CAS: 18) | 37 |
| Total | 45 | 50 | 95 |

ECNU: East China Normal University, Shanghai, China; SSTM: Shanghai Science and Technology Museum, China; CAS: Chinese Academy of Sciences, Beijing, China

Table 14. Measurements of crania. Numbers correspond measurements shown in Fig. 9.

| Abbreviation | Variable |
|---|---|
| Cra01 | Greatest length of the skull |
| Cra02 | Condylobasal length |
| Cra03 | Basal length |
| Cra04 | Short skull length |
| Cra05 | Premolar – Prosthion |
| Cra06 | Basicranial axis |
| Cra07 | Basifacial axis |
| Cra08 | Neurocranium length |
| Cra09 | Viscerocranium length |
| Cra10 | Median frontal length |
| Cra11 | Lambda – Nasion |
| Cra12 | Lambda – Rhinion |
| Cra13 | Lambda – Prosthion |
| Cra14 | Akrokranion – Infraorbitale of one side |
| Cra15 | Greatest length of the nasals |
| Cra16 | Snout length |
| Cra17 | Median palatal length |
| Cra18 | Oral palatal length |
| Cra19 | Lateral length of the premaxilla |
| Cra20 | Length of the molar and premolar row |
| Cra21 | Length of the molar row |
| Cra22 | Length of the premolar row |
| Cra23 | Length of the orbit |
| Cra24 | Length of the orbit |
| Cra25 | Greatest mastoid breadth |
| Cra26 | Greatest breadth of the occipital condyles |
| Cra27 | Greatest breadth at the bases of the paraoccipital processes |
| Cra28 | Greatest breadth of the foramen magnum |
| Cra29 | Greatest height of the foramen magnum |
| Cra30 | Least frontal breadth |

Table 14. continued.

| | |
|---|---|
| Cra31 | Zygomatic breadth |
| Cra32 | Least breadth between the orbits |
| Cra33 | Greatest breadth across the orbits |
| Cra34 | Greatest breadth across the nasals |
| Cra35 | Greatest breadth across the premaxillae |
| Cra36 | Basion – the highest point of the superior nuchal crest |

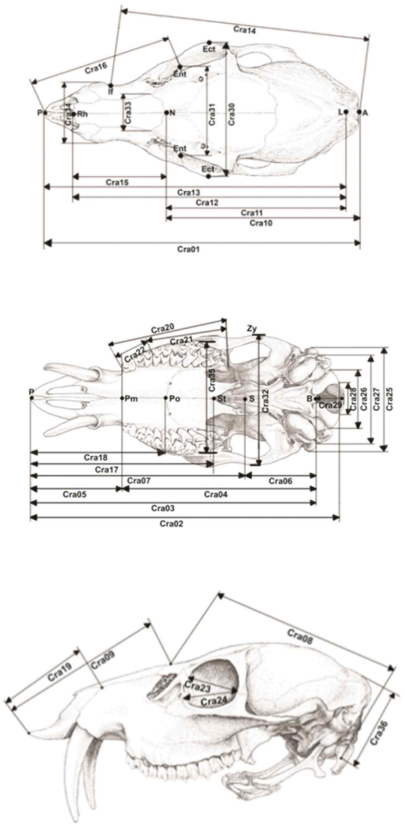


Fig. 9. Diagram of craniofacial measurements. A: Akrokranion, B: Basion, Ect: Ectorbitale, Ent: Entorbitale, If: Infraorbitale, L: Lambda, N: Nasion, P: Prosthion, Pm: Premolare, Po: Palatinoorale, Rh: Rhinion, S: Synsphenion, St: Staphylion, and Zy: Zygion.

## RESULTS

According to the Student's *t*-test between the two subspecies, seven skull measurements from female specimens (Cra7, Cra20, Cra26, Cra27, Cra30, Cra33, and Cra36) and male specimens (Cra15, Cra20, Cra22, Cra27, Cra28, Cra30, and Cra36) were significantly larger for *H. i. argyropus* than *H. i. inermis* (Table 15). In contrast, one measurement from females (Cra06) and from males (Cra34) was significantly larger for *H. i. inermis* than *H. i. argyropus*. In addition, most average values for all other measurements, which were not significantly different between subspecies, were larger in *H. i. argyropus* than *H. i. inermis* in females. Skull length (Cra01) was significantly larger in females than males in both populations (Table 15). Among all measurements, 14 and 17 traits were significantly larger for females from China and Korea, respectively.

Table 15. Mean (in mm) ± standard deviation (SD) of measurements.

| Measurement | *H. i. argyropus* | | | | *H. i. inermis* | | | |
|---|---|---|---|---|---|---|---|---|
| | Male | | Female | | Male | | Female | |
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Geometric mean | 56.15 | | 57.1 | | 55.85 | | 56.12 | |
| Cra01 | 168.33*** | 4.87 | 173.26 | 3.56 | 169.22** | 3.96 | 172.54 | 4.78 |
| Cra02 | 158.24*** | 5.19 | 163.13 | 3.31 | 158.59** | 3.67 | 162.25 | 4.79 |
| Cra03 | 147.79*** | 4.99 | 152.87 | 3.41 | 148.62** | 3.50 | 152.07 | 4.52 |
| Cra04 | 94.36*** | 2.34 | 97.15 | 1.96 | 94.29* | 2.43 | 96.37 | 3.39 |
| Cra05 | 53.39* | 3.07 | 55.67 | 2.65 | 54.24 | 1.92 | 55.44 | 2.35 |
| Cra06 | 36.64* | 1.62 | 37.82 | 1.93 | 36.99*** | 2.11 | **39.91** | 3.68 |
| Cra07 | 113.31*** | 3.81 | 117.11 | 3.32 | 113.81 | 3.60 | 114.16 | 4.35 |
| Cra08 | 93.21 | 3.33 | 94.58 | 1.99 | 93.78 | 3.41 | 93.18 | 3.74 |
| Cra09 | 81.26** | 3.56 | 84.79 | 3.39 | 81.29** | 3.06 | 84.05 | 3.17 |
| Cra10 | 93.80 | 3.23 | 95.10 | 2.17 | 94.54 | 3.20 | 94.38 | 3.99 |
| Cra11 | 83.25 | 3.09 | 83.68 | 2.25 | 83.32 | 3.35 | 83.57 | 4.09 |

Table 15. continued.

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Cra12 | 135.56* | 3.86 | 138.02 | 3.84 | 133.34* | 4.76 | 135.93 | 3.80 |
| Cra13 | 160.67** | 4.80 | 164.73 | 3.95 | 160.73** | 4.31 | 164.22 | 4.77 |
| Cra14 | 117.23*** | 3.08 | 120.14 | 1.87 | 117.29* | 3.22 | 119.44 | 4.01 |
| Cra15 | **52.91** | 3.50 | 54.99 | 3.37 | 50.93* | 3.93 | 53.10 | 3.39 |
| Cra16 | 80.67*** | 2.82 | 84.15 | 2.95 | 81.13** | 2.63 | 83.48 | 2.96 |
| Cra17 | 95.61*** | 4.38 | 100.18 | 3.36 | 97.40* | 3.54 | 99.56 | 2.97 |
| Cra18 | 72.40** | 3.46 | 75.38 | 2.92 | 73.20 | 2.31 | 74.47 | 2.71 |
| Cra19 | 46.72* | 3.06 | 49.04 | 3.16 | 46.69 | 3.00 | 48.34 | 2.64 |
| Cra20 | **50.17** | 2.22 | **49.91** | 2.40 | 48.84 | 1.96 | 48.32 | 2.29 |
| Cra21 | 27.98 | 1.11 | 27.87 | 1.46 | 27.90 | 1.35 | 27.45 | 1.47 |
| Cra22 | **23.77** | 1.57 | 23.82 | 1.54 | 22.97 | 1.04 | 22.89 | 1.25 |
| Cra23 | 25.44* | 1.04 | 26.05 | 0.86 | 25.51 | 0.79 | 25.86 | 1.08 |
| Cra24 | 25.24 | 1.22 | 25.26 | 1.00 | 24.99 | 0.93 | 25.13 | 0.95 |
| Cra25 | 47.28 | 1.62 | 47.45 | 1.99 | 47.39 | 1.93 | 46.93 | 1.78 |
| Cra26 | 29.21 | 2.41 | **29.28** | 0.87 | 28.21 | 1.21 | 28.13 | 1.15 |
| Cra27 | **41.87** | 1.27 | **42.25** | 1.67 | 40.81* | 1.57 | 39.89 | 1.6 |
| Cra28 | 14.39 | 0.83 | 14.06 | 0.95 | 13.86 | 0.83 | 14.08 | 1.18 |

Table 15. continued.

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Cra29 | 14.87 | 0.82 | 14.59 | 1.30 | 14.64 | 0.94 | 14.97 | 1.16 |
| Cra30 | **71.97** | 2.66 | **73.32** | 2.16 | 70.12 | 2.66 | 69.22 | 3.06 |
| Cra31 | 39.25 | 1.91 | 40.60 | 3.02 | 38.22 | 2.21 | 39.39 | 2.01 |
| Cra32 | 71.20 | 2.62 | 71.94 | 2.45 | 71.11 | 2.64 | 71.53 | 3.13 |
| Cra33 | 16.29 | 1.74 | **16.76** | 1.54 | 16.24 | 2.35 | 15.29 | 1.52 |
| Cra34 | 29.59*** | 2.45 | 26.80 | 2.56 | **31.28***** | 2.12 | 25.93 | 1.86 |
| Cra35 | 51.43 | 2.18 | 52.70 | 2.01 | 51.69 | 1.72 | 51.44 | 2.08 |
| Cra36 | **41.72** | 1.54 | **42.07** | 1.30 | 40.92 | 1.48 | 40.82 | 1.59 |

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$; bold: significant difference between Korea and China.

In the FA of cranium measurements, the first (F1) and second (F2) factor axes explained 41.81% and 26.56% of the total variation in males (Fig. 10A), and 32.50% and 14.31% of the variation in females (Fig. 10B). The reliability of this analysis as tested by standardized Cronbach's alpha was 0.94 in males and 0.92 in females (Table 17). Therefore, the reliability of the results was accepted as fairly high. The loading factor values are presented in Table 17. In general, the F1 loadings reflected skull length measurements in both males and females. In the DA, most of the individuals were allocated to their original population. Fig. 11 is a scatter plot of the DA between the two populations. However, some were allocated to the wrong cluster, or allocated to more than two populations (Table 18). For example, 20672, 1742 and 17911 were allocated to the Korean cluster, although these specimens are from the Chinese population. Similarly, KJ0120, KJ0009 and KJ0003 were allocated to the correct population (Korea) but their probabilities of being assigned to the Chinese population were high at 14.9%, 13.6% and 34.7%, respectively. PCA was conducted for

males and females separately, and the scatter plots seemed to be consistent with the “75% rule” for both sexes. Fig. 12A is a scatter plot of the PCA of females between the two populations and Fig. 12B is that of males.

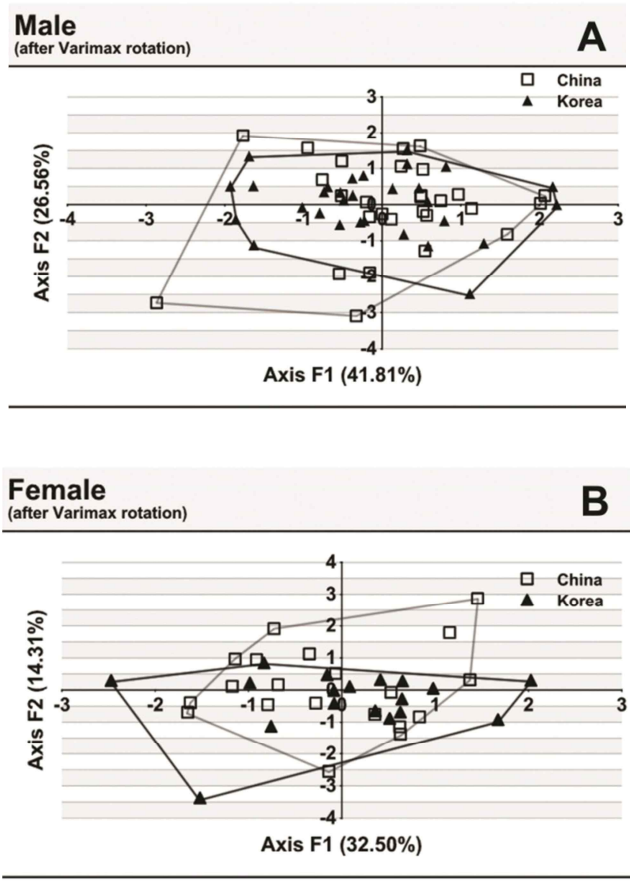


Fig. 10. Factor reduction analysis plots of male skulls (A) and female skulls (B) of the Korean population (closed triangles) and Chinese population (open squares).

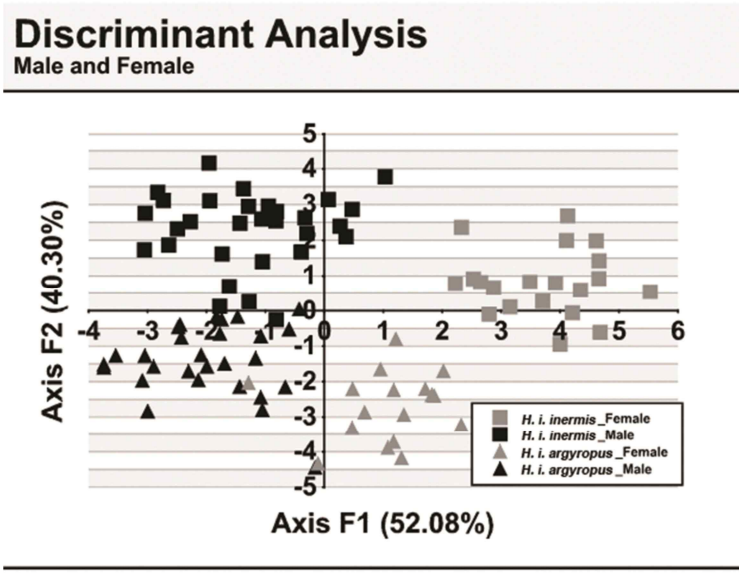


Fig. 11. Discriminant analysis plot of Korean population (closed triangle) and Chinese population (open squares).

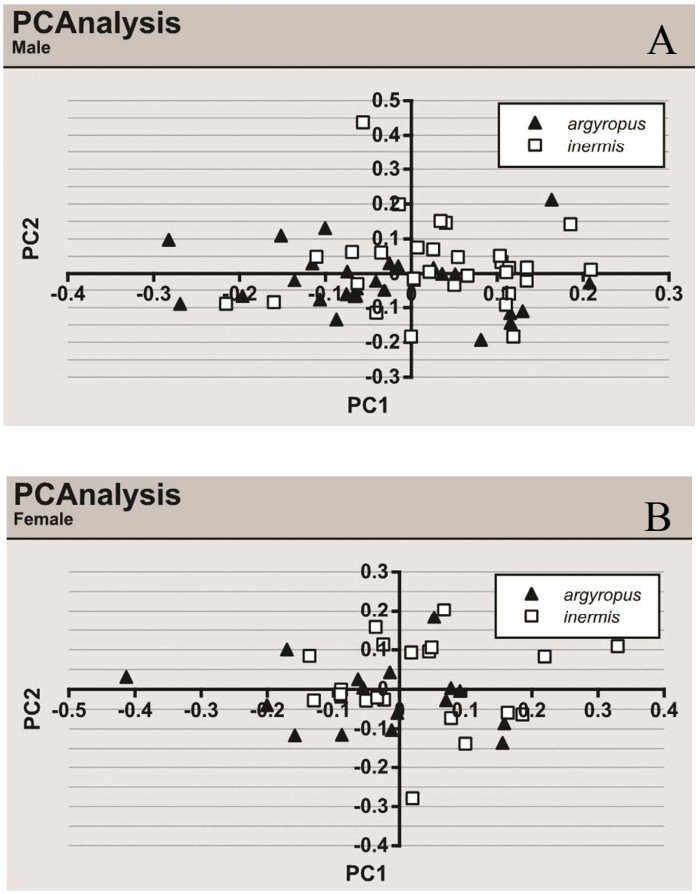


Fig. 12. Principal component analysis plot of male (A) and female (B) of Korean population (closed triangle) and Chinese population (open squares).

Table 16. Pairwise morphological distance matrix.

| **Male** | *argyropus* | *inermis* |
|---|---|---|
| *argyropus* | 0.972 | |
| *inermis* | 0.971 | 0.972 |

| **Female** | *argyropus* | *inermis* |
|---|---|---|
| *argyropus* | 0.973 | |
| *inermis* | 0.970 | 0.971 |

Table 17. Factor loading values for each measurements, Standardized Cronbach's alpha, eigenvalues, contribution rates, and cumulative rates in principal component analyses.

| **Cranium** | | | | |
|---|---|---|---|---|
| Measurement point | Male | | Female | |
| | F1 | F2 | F1 | F2 |
| Cra01 | 0.78 | -0.54 | 0.89 | -0.01 |
| Cra02 | 0.80 | -0.47 | 0.88 | -0.07 |
| Cra03 | 0.81 | -0.53 | 0.89 | -0.11 |
| Cra04 | 0.52 | -0.68 | 0.59 | 0.28 |
| Cra05 | 0.86 | -0.23 | 0.77 | -0.44 |
| Cra06 | 0.16 | -0.46 | 0.11 | -0.32 |
| Cra07 | 0.83 | -0.35 | 0.70 | 0.19 |
| Cra08 | 0.13 | -0.91 | 0.28 | 0.34 |
| Cra09 | 0.95 | 0.15 | 0.89 | -0.05 |
| Cra10 | 0.11 | -0.93 | 0.28 | 0.23 |
| Cra11 | 0.15 | -0.85 | 0.34 | 0.16 |
| Cra12 | 0.59 | -0.26 | 0.76 | 0.28 |
| Cra13 | 0.79 | -0.48 | 0.90 | -0.01 |
| Cra14 | 0.53 | -0.64 | 0.63 | 0.14 |
| Cra15 | 0.57 | 0.33 | 0.48 | 0.19 |
| Cra16 | 0.85 | -0.23 | 0.88 | -0.11 |
| Cra17 | 0.84 | -0.13 | 0.78 | -0.17 |
| Cra18 | 0.79 | -0.19 | 0.76 | -0.3 |
| Cra19 | 0.56 | 0.05 | 0.60 | -0.08 |

Table 17. continued.

| | | | | |
|---|---|---|---|---|
| Cra20 | 0.01 | 0.01 | -0.11 | 0.91 |
| Cra21 | 0.07 | -0.13 | -0.26 | 0.85 |
| Cra22 | -0.19 | 0.11 | -0.11 | 0.76 |
| Cra23 | 0.20 | -0.28 | 0.15 | -0.14 |
| Cra24 | 0.33 | -0.26 | 0.43 | 0.08 |
| Cra25 | 0.15 | -0.52 | 0.12 | -0.02 |
| Cra26 | 0.30 | -0.19 | 0.15 | 0.43 |
| Cra27 | 0.15 | -0.13 | 0.17 | 0.21 |
| Cra28 | -0.03 | -0.19 | -0.08 | 0.02 |
| Cra29 | -0.17 | -0.07 | 0.13 | 0.21 |
| Cra30 | 0.15 | -0.20 | 0.19 | -0.06 |
| Cra31 | 0.01 | -0.19 | 0.03 | 0.11 |
| Cra32 | 0.31 | -0.09 | -0.12 | -0.30 |
| Cra33 | 0.24 | 0.15 | 0.16 | 0.05 |
| Cra34 | 0.08 | -0.04 | 0.06 | -0.05 |
| Cra35 | 0.29 | -0.18 | 0.04 | -0.02 |
| Cra36 | 0.28 | -0.40 | 0.28 | 0.49 |
| Standardized Cronbach's alpha: | 0.94 | | 0.92 | |
| Eigenvalues | 13.69 | 3.43 | 11.59 | 4.93 |
| Contribution rates | 0.38 | 0.10 | 0.32 | 0.14 |
| Cumulative contribution rates | 0.38 | 0.48 | 0.32 | 0.46 |

The within-population and inter-population morphological similarities computed by the Euclidean method were estimated for both sexes (Table 16). For males, the intra-population morphological similarity was 0.972 for both populations, and the inter-population distance was 0.971. For females, the intra-population similarity was 0.973 for the Korean population and 0.971 for the Chinese population. The inter-population similarity was 0.970. Figs 13 and 14 show the results of cluster analysis using the UPGMA and NJ methods, respectively, as well as the cladogram topologies. Both methods showed that specimens from each population had mixed topologies in two cladograms; the morphological distance (=similarity, Y-axis) was <1% in the UPGMA cladogram.

Table 18. Result of discriminant analysis (Prob.=probability; Prior=original assignment, Post=re-assignment; M=male, F=female).

| Specimen | Prior | Post | Prob. Korea_F | Prob. Korea_M | Prob. China_F | Prob. China_M |
|---|---|---|---|---|---|---|
| KJ0149 | Korea_M | Korea_M | 0 | 0.964 | 0 | 0.036 |
| KJ0147 | Korea_M | Korea_M | 0.006 | 0.976 | 0 | 0.018 |
| KJ0146 | Korea_M | Korea_M | 0.002 | 0.967 | 0 | 0.031 |
| KJ0135 | Korea_M | Korea_M | 0.087 | 0.906 | 0 | 0.007 |
| KJ0120 | Korea_M | Korea_M | 0.111 | 0.740 | 0 | 0.149 |
| KJ0117 | Korea_M | Korea_F | 0.969 | 0.031 | 0 | 0 |
| KJ0114 | Korea_M | Korea_M | 0.058 | 0.942 | 0 | 0 |
| KJ0063 | Korea_M | Korea_M | 0.012 | 0.988 | 0 | 0 |
| KJ0020 | Korea_M | Korea_M | 0 | 0.977 | 0 | 0.023 |
| KJ0014 | Korea_M | Korea_M | 0.012 | 0.988 | 0 | 0 |
| KJ0009 | Korea_M | Korea_M | 0.001 | 0.863 | 0 | 0.136 |

Table 18. continued.

| | | | | | | |
|---|---|---|---|---|---|---|
| KJ0003 | Korea_M | Korea_M | 0.011 | 0.641 | 0.001 | 0.347 |
| 20672 | China_M | Korea_M | 0.041 | 0.633 | 0 | 0.326 |
| Beijing02 | China_M | China_M | 0 | 0.014 | 0 | 0.986 |
| 1742 | China_M | Korea_M | 0 | 0.856 | 0 | 0.144 |
| Beijing13 | China_M | Korea_M | 0.001 | 0.638 | 0 | 0.362 |
| 17911 | China_M | China_M | 0.003 | 0.226 | 0 | 0.771 |
| KJ0164 | Korea_F | Korea_F | 0.961 | 0.039 | 0 | 0 |
| KJ0160 | Korea_F | Korea_F | 0.923 | 0.072 | 0.005 | 0 |
| KJ0121 | Korea_F | Korea_F | 0.667 | 0.332 | 0 | 0.001 |
| KJ0058 | Korea_F | Korea_F | 0.930 | 0.021 | 0.044 | 0.005 |
| 3670 | China_F | China_F | 0.161 | 0 | 0.839 | 0 |
| H1216 | China_F | China_F | 0 | 0 | 0.99 | 0.01 |

Prob.: Probability.

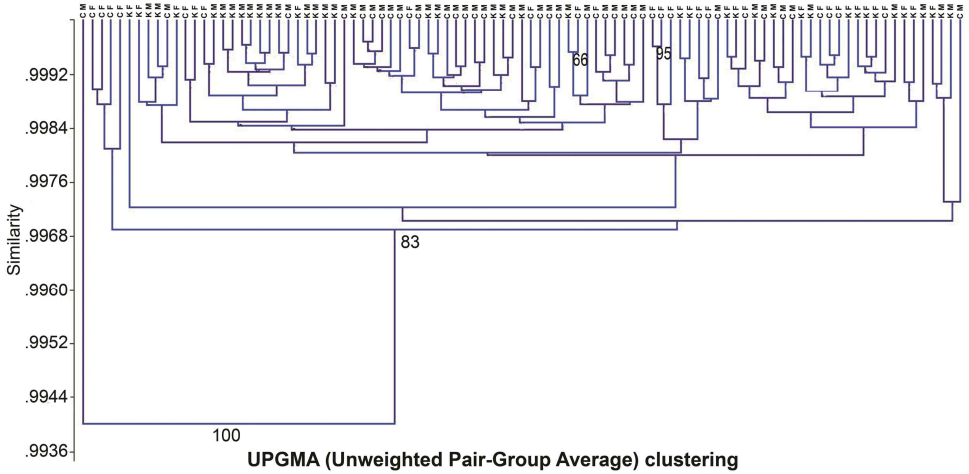


Fig.13. UPGMA clustering diagram of the Korean population and Chinese population. (C: Chinese population; K: Korean population; M: male; F: female).

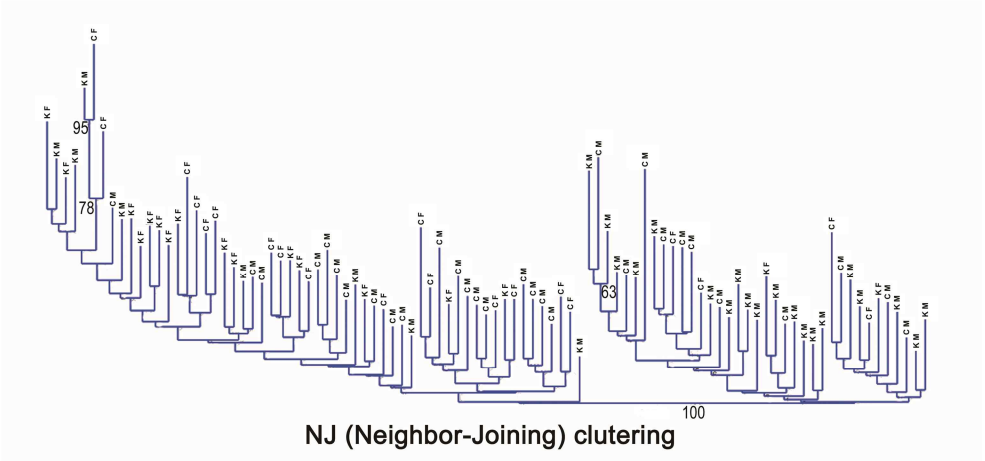


Fig. 14. NJ clustering diagram of the Korean population and Chinese population. (C: Chinese population; K: Korean population; M: male; F: female).

# DISCUSSION

## Morphological differences between *H. i. inermis* and *H. i. argyropus*

The present study investigated the two subspecies of water deer (*H. inermis*) distributed in Korea and China. The major goal of this research was to morphologically test the conventional subspecific classification of this species. The results of a Student's *t*-test and FA suggested that these two subspecies are not well-differentiated, meaning that individuals of the two populations share common morphological traits. DA results indicated that some individuals share characteristics of both populations, suggesting that not all individuals can be assigned to their original population based on morphometrics. In particular, DA revealed four specimens that were not assigned to their correct population based on linear dimensions: three male specimens of *H. i. inermis* were assigned as *H. i. argyropus* males, and one male specimen of *H. i. argyropus* was assigned as a female *H. i. argyropus*. The results of cluster analysis

using two different algorithms, NJ and UPGMA, showed that specimens of the two populations (China and Korea) had highly mixed topologies in the cladograms.

Previous research has confirmed that the two subspecies have a close genetic distance, with an average genetic distance of 2.1% in the control region and 1.3% in cytochrome *b* (Koh et al., 2009). The topology of a phylogenetic NJ tree from the control region and cytochrome *b* showed that *H. i. inermis* and *H. i. argyropus* blended within a major clade. In the present study, it was found that the morphological distance between the populations was also very close. The inter-population similarity was almost the same as the intra-population similarity. These facts suggest that the two populations can not be clearly distinguished genetically nor morphologically.

Bergmann's rule posits that body size is negatively correlated with temperature among closely related species in mammals and birds (Bergmann, 1847; James, 1970). This pattern has been pointed out to be obvious, especially within species (Mayr, 1956, 1963) and has

been regarded as one of the major factors producing within-species geographic variation (Ridley, 1996; Futuyma, 2009). However, our results show that water deer do not follow Bergmann's rule. Cra1, which represents skull size, was not significantly different between *H. i. inermis* and *H. i. argyropus* (Table 15). The Zhoushan Islands, the habitat of the Chinese water deer in central China (Fig. 8), are located around 30°N latitude, and Korean water deer are distributed around 35–38°N latitude. Similar to the present results, others (for example, red fox) have demonstrated that this rule is not always applicable (e.g., Ashton et al., 2000; Dayan et al., 1991; McNab, 1971; Oishi et al., 2010; Takeuchi, 1995; Uraguchi, 2009). Although the reason for the lack of clear morphocline in water deer is yet unclear, the establishment of current distribution of this species was perhaps a relatively recent event, producing the observed genetic and morphological homogeneity.

**Necessity to reconsider the subspecies classification of water deer**

Subspecies of water deer were initially designated by Swinhoe (1870) and Heude (1884). Although the concept of subspecies has varied, it is generally defined as members of a polytypic species, not simply as a "slightly different" local population (Mayr, 1982). Results of PCA indicate that the two populations show more than 75% of overlap and reject the subspecies classification under the "75% rule (Amadon, 1949)". The Amadon's rule or 75% rule defines that two populations belong to the different subspecies if the 75% of individuals in a population A is separable by same character from all members of an overlapping population B (Amadon, 1949). Phylogenetic analysis studying the mtDNA proposed that the subspecific classification may not be valid, and indicated the need for examination of this issue from a morphological perspective (Koh et al., 2009). Our results demonstrate that there is no clear difference in craniodental morphology between the two populations, lending

further support to the reconsideration of the subspecific classification of water deer. The differences between the Chinese and Korean populations are thus not exceptional, other than their pelage color (Tate, 1940). However, the pelage color variation has not been studied quantitatively and remains to be evaluated (see Koyabu et al., 2008).

Although the Chinese water deer was originally distributed widely throughout China, these animals have gradually become rarer, and their distribution has been fragmented over the past century due to poaching for traditional medicine and habitat destruction by industrialization (Zhang, 1996; Wang, 1998; Xu et al., 1998; Harris and Duckworth, 2008). Currently, the Chinese water deer is classified as a vulnerable species by the International Union for Conservation of Nature (IUCN) (Harris and Duckworth, 2008). In contrast to the situation in China, Korean water deer are distributed throughout the Korean Peninsula, where its numbers are both stable and abundant (Won and Smith, 1990). If the population of Chinese

water deer continues to decrease, plans for restoration, conservation and management of the Chinese water deer will become more urgent. Given the homogeneity of Chinese and Korean water deer demonstrated by molecular evidence (Koh et al., 2009) and morphological evidence (this study), the introduction of Korean water deer (*H. i. argyropus*) into the Chinese population might be the most logical, and ultimately successful, restoration strategy.

# CHAPTER II: Genetic Studies of the Korean Water Deer, *Hydropotes inermis argyropus*

## Part IV: Genetic Status and Population Structure of the Korean Water Deer, *Hydropotes inermis argyropus*

### INTRODUCTION

The subfamily, Hydropotinae, is represented by a single species: *Hydropotes inermis*, the water deer (Putman, 1988). Taxonomically, this species is consisted of two subspecies, the Chinese water deer, *H. i. inermis*, which is native to China, and the Korean water deer, *H. i. argyropus*, which is endemic to the Korean Peninsula (Cooke and Farrell, 1998).

Although the Korean water deer is an important species as an endemic biological resource in South Korea, the genetic studies such as gene flow, genetic diversity, population structure and genetic structure have not been studied well. Recently, several fundamental

studies of the Korean water deer have been reported. For example, sexual dimorphism and growth pattern based on craniomandibular traits were studied, and the genital organ anatomy of the male and the lamination of the masseter muscle were also observed (Kim et al., 2013(a), (b); Sasaki et al., 2013; Sohn and Kimura, 2013). For the Chinese water deer, several genetic studies have been executed. Using 403 base pair fragment of the mitochondrial DNA control region, Hu et al., (2006) reported the genetic diversity and population structure of the Chinese water deer. In Hu′s study, 18 haplotypes in 40 samples were detected and the genetic diversity of this species was higher than other rare cervid species. Hu et al. (2007) reported that genetic diversity of captive zoo population is higher than that of the two wild populations using 7 microsatellite markers derived from bovine. Koh et al. (2009) reported the phylogenetic relationship between the Chinese water deer and the Korean water deer using mitochondrial DNA cytochrome *b* and control region. In Koh′s study, genetic distances between two

subspecies was 2.1% in the cytochrome *b* region and 1.3% in the control region, and two sympatric phylogroups of the Korean water deer were identified. Lee et al. (2011) isolated 12 microsatellite markers from the Korean water deer, and reported that there were no significant regional or genetic structure differences between the mid-eastern and southwestern populations in South Korea.

In this dissertation, mitochondrial cytochrome *b* gene was used and tried to investigate the genetic diversity in populations of Korean water deer. This sequence has been used in molecular phylogenetic researches in many other animal groups (Birungi and Arctander, 2001; Chikuni et al., 1995; Groves andShields, 1996; Hassanin et al., 1998; Honeycutt et al., 1995; Irwin et al., 1991; Matthee and Robinson, 1999). Furthermore, I determined the genetic structure of the three populations (Northern, Central and Southern) of the Korean water deer using 12 microsatellite markers, as supplementary to the previous research (Lee et al., 2011).

## MATERIALS AND METHODS

**Sample collection and DNA extraction:** A total of 53 Korean water deer samples (muscle tissue) were collected in three provinces of Korean Peninsula, northern (Gangwon), central (Daejeon) and southern region (Jeonnam) (Fig. 15). All the samples were stored at -70ºC and genomic DNA was extracted from the tissue using DNeasy Tissue Kit (Qiagen, Valencia, CA) following the manufacturer's instructions.

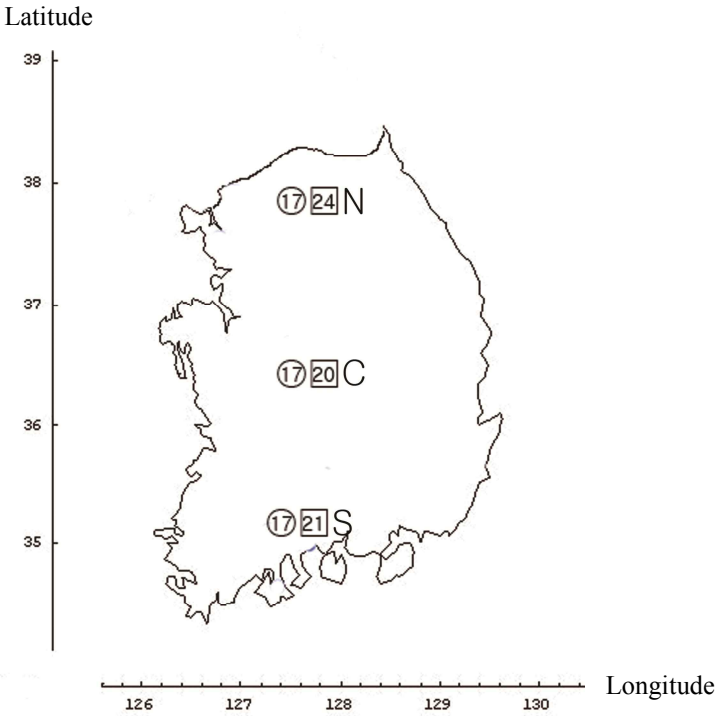


Fig. 15. Collection sites for Korean water deer used in this study. The three sites in Korea: N = Northern, C = Central and S = Southern. ○: numbers of samples for cytochrome *b* region and □: numbers of samples for microsatellite markers.

**MtDNA sequencing and microsatellite genotyping:** The mtDNA cytochrome *b* region was amplified with a pair of primers, L14724 (5'- CGA AGC TTG ATA TGA AAA ACC ATC GTT G) and H15915 (5'- AAC TGC AGT CAT CTC CGG TTT ACA AGA C) (Irwin et al., 1991). PCR (Polymerase Chain Reaction) amplification was performed in 30μl of reactions containing 25ng total genomic DNA, 1.5mM $MgCl_2$, 200μM of each dNTP, 0.3mM of each primer, 0.5 unit of *Taq* polymerase using following PCR condition: an initial denaturation for 4 min at 94ºC, followed by 35 cycles (94ºC for 30s, 45ºC for 40s, and 72ºC for 60s) with a final extension for 5 min at 94ºC. PCR products were resolved by electrophoresis on ethidium bromide stained 1% agarose gel, and visualized under ultraviolet illumination.

For microsatellite analysis, 12 primer sets, which are developed for water deer were used (Lee et al., 2011). PCR was carried out in 10 μl reaction volume containing 10-50 ng of template DNA, 1x PCR buffer, 0.2 mM of each dNTP, 0.3 μm of each primer and 0.5 unit of

*Taq* polymerase. In this study, I followed PCR condition of the previous study (Lee et al., 2011). Touchdown amplification was performed, initial denaturation at 94ºC for 3 min, followed by 20 cycles at 94ºC for 1 min, annealing from 60ºC to 50ºC decreased by 0.5ºC per cycle, 72ºC for 1 min, and followed by 20 cycles at 94ºC for 1 min, 50ºC for 1min, and 72ºC for 1 min, and a final extension at 72ºC for 10min.

**Data analysis:** Mitochondrial DNA cytochrome *b* region sequences (1140 bp) were aligned with Clustal X version 1.83 (Tompson et al., 1997). Haplotype (*h*) and nucleotide ($\pi$) diversities were calculated using DnaSP v5 (Librado and Rozas, 2009). Neighbor-joining method was used to reconstruct phylogenetic relationship among haplotypes using the program MEGA version 4 (Tamura et al., 2007). Bootstrapping (1000 replicates) were applied to assess the relative contribution for branches and two mtDNA cytochrome *b* region of the roe deer, *Capreolus capreolus* (GenBank accession nos. AJ00024 and Y14951), were chosen as an outgroup (Doozery and

Randi, 1997; Shi et al., 2004).

Test for deviation from Hardy-Weinberg Equilibrium (HWE) was conducted for each locus using GENEPOP version 4.2 (Rousset, 2008) and the number of alleles per locus and expected ($H_E$) and observed ($H_O$) heterozygosities were used to estimate genetic diversity in each population using CERVUS version 3.0 program (Marshall et al., 1998). To quantify the extent of genetic differentiation among the 3 populations, $F_{ST}$ and $F$ statistics per locus among populations and their statistical $p$-value were calculated using GENEPOP version 4.2 (Rousset, 2008). The program STRUCTURE 2.3.3 was used to demonstrate the population structure of this species (Pritchard et al., 2000) and the statistical certainty of assignment for individuals into their original population was evaluated using GENECLASS 2.0 program (Piry et al., 2004).

## RESULTS

**Genetic variability of the Korean water deer:** The mtDNA cytochrome *b* region (1140 bp in length) was analyzed for 51 Korean water deer. For the 3 geographic populations studied, a total of 21 haplotypes were defined by 42 polymorphic sites (Table 19). The overall haplotype diversity and nucleotide diversity of cytochrome *b* region were 0.776 and 0.564%, respectively. Among 3 geographic populations, the number of haplotype and polymorphic site of the northern part were 0.926 and 0.633%, respectively, and it is higher than the others (Table 19). There were 5 shared haplotypes among 3 populations. Three haplotypes were shared by 3 populations and two haplotypes were shared by central and northern populations. These haplotypes were defined by 17 variable sites with 19 substitutions (13 transitions and 6 transversions) (Table 20). Among haplotypes, Hap02 and Hap11 were the most common, and shared by 16 and 18 individuals, respectively. The unique haplotypes of Northern population were 6 (Hap 03, Hap05, Hap06,

Hap08, Hap09 and Hap13), those of Southern population were 2 (Hap07 and Hap10) and Central population did not have any unique haplotype.

Table 19. Mitochondrial DNA cytochrome *b* diversity of the Korean water deer in this study based on 1140 base pair.

| | N | No. of Haplotype | No. of Polymorphic site | Haplotype diversity (SD) | % Nucleotide diversity |
|---|---|---|---|---|---|
| Northern | 17 | 11 | 17 | 0.926 (0.045) | 0.633 |
| Central | 17 | 5 | 13 | 0.625 (0.012) | 0.508 |
| Southern | 17 | 5 | 12 | 0.581 (0.017) | 0.337 |
| Total | 51 | 21 | 42 | 0.776 (0.002) | 0.564 |

Table 20. Sequence variations of the mtDNA cytochrome *b* region haplotypes in Korean water deer

| | Variable sites | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Haplotype (N) | 7 | 8 | 393 | 450 | 478 | 573 | 618 | 663 | 720 | 804 | 887 | 915 | 970 | 1035 | 1087 | 1093 | 1138 |
| Hap01 (5) | A | C | T | G | C | T | T | T | G | T | C | C | T | T | C | C | A |
| Hap02 (16) | . | . | C | A | T | A | C | C | A | . | . | T | C | C | . | T | . |
| Hap03 (1) | G | . | C | A | T | A | C | C | A | . | . | T | C | C | . | T | . |
| Hap04 (2) | . | . | C | A | T | A | C | C | A | C | . | T | C | C | . | T | . |
| Hap05 (1) | . | . | . | . | . | . | . | . | . | . | . | . | . | . | G | . | . |
| Hap06 (1) | G | . | C | A | T | A | C | C | A | C | . | T | C | C | . | T | T |
| Hap07 (1) | . | G | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| Hap08 (1) | G | . | C | A | T | A | C | C | A | . | T | T | C | C | . | T | T |

Table 20. continued.

| | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Hap09 (1) | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| Hap10 (1) | . | . | C | A | T | . | C | C | A | . | . | T | C | C | . | A | . |
| Hap11 (18) | . | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| Hap12 (2) | . | . | . | . | . | . | . | . | . | . | . | . | C | . | . | . | . |
| Hap13 (1) | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |

Variable base pairs are shown. Dots are the same base pair with the first sequence. n: Number of indivisuals having the same haplotypes.

**Genetic relationship, population structure and individual assignment:** The neighbor-joining tree showed that mtDNA cyt-*b* sequences form two clades (Clades 1 and 2), however these two clades were not formed by regional division (Fig. 16). Clades 1 and 2 were represented by mixture of individuals of 3 regional populations. Structure analysis showed that the posterior likelihood value was the highest, when K was set to 1 (Fig. 17). Assignment test using the program GENECLASS showed that individuals from 3 populations could not display a tendency of differentiation following their population origin. The ratio of correctly assigned individuals was only 35.4% (n=23) and 22 individuals in Northern population and all individuals of Southern and Central populations were assigned more than two groups, simultaneously.

As a whole, 73 alleles were observed from 12 microsatellite loci (Table 21). Average allele numbers per locus was 5.42, 4.25 and 4.92 for Northern, Central and Southern, respectively. In Northern population, 5 loci (Hi03, Hi05, Hi06, Hi07 and Hi10) were the least

polymorphic with 2 alleles, and Hi15 was the most polymorphic with 12 alleles. In Central population, 4 loci (Hi03, Hi05, Hi06 and Hi07) were the least polymorphic with 2 alleles, and Hi13 was the most polymorphic with 8 alleles. In Southern population, 3 loci (Hi03, Hi05 and Hi07) were the least polymorphic with 2 alleles, and Hi35 was the most polymorphic with 10 alleles. Totally, Hi03, Hi05 and Hi07 were the least polymorphic loci with only 2 alleles, and Hi15 was the most polymorphic locus with 13 alleles.

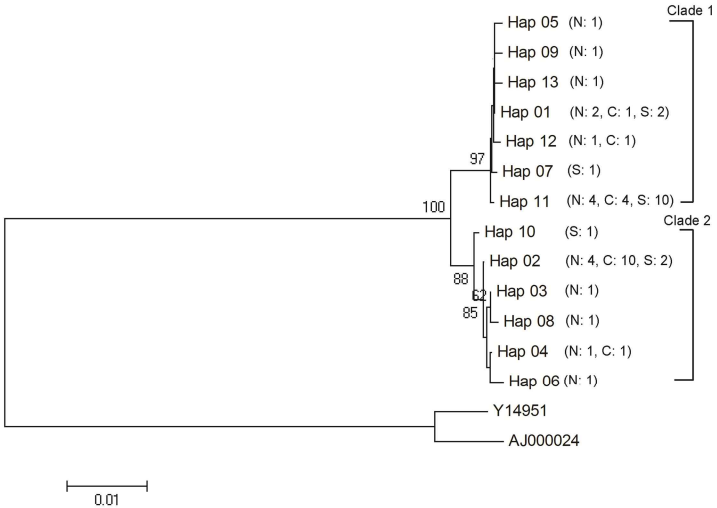


Fig. 16. Phylogenetic relationship among 3 populations of the Korean water deer by the neighbor-joining tree of mtDNA cytochrome *b* sequences (N = Northern, C = Central and S = Southern). Numbers at the branches denote the bootstrap values. Outgroup: Roe deer, *Capreolus capreolus* (AJ00024 and Y14951).

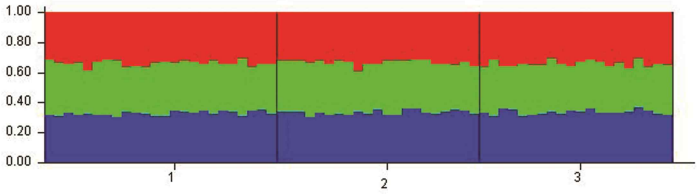


Fig. 17. Bar plot (K = 1) from population structure analysis for Korean water deer.

Table 21. Descriptive statistics of the Korean water deer populations.

| Locus | Northern (Gangwon) | | | | | Central (Daejeon) | | | | | Southern (Neonnam) | | | | | Total | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | A | $H_E$ | $H_O$ | P | N | A | $H_E$ | $H_O$ | P | N | A | $H_E$ | $H_O$ | P | N | A | $H_E$ | $H_O$ | P | $F_{ST}$ |
| Hi02 | 24 | 3 | 0.646 | 0.583 | ns | 20 | 4 | 0.661 | 0.684 | ns | 21 | 4 | 0.692 | 0.429 | ns | 64 | 4 | 0.669 | 0.563 | ns | 0.002 |
| Hi03 | 24 | 2 | 0.383 | 0.167 | * | 20 | 2 | 0.422 | 0.158 | * | 21 | 2 | 0.438 | 0.429 | ns | 64 | 2 | 0.407 | 0.250 | ** | -0.029 |
| Hi05 | 24 | 2 | 0.223 | 0.167 | ns | 20 | 2 | 0.053 | 0.053 | ns | 21 | 2 | 0.251 | 0.190 | ns | 64 | 2 | 0.184 | 0.141 | ns | 0.011 |
| Hi06 | 24 | 2 | 0.496 | 0.583 | ns | 20 | 2 | 0.514 | 0.556 | ns | 21 | 4 | 0.612 | 0.476 | * | 63 | 4 | 0.542 | 0.540 | ns | 0.006 |
| Hi07 | 24 | 2 | 0.403 | 0.542 | ns | 20 | 2 | 0.193 | 0.211 | ns | 21 | 2 | 0.345 | 0.429 | ns | 64 | 2 | 0.326 | 0.406 | ns | 0.026 |
| Hi08 | 24 | 8 | 0.743 | 0.625 | ns | 20 | 5 | 0.727 | 0.650 | ns | 21 | 7 | 0.804 | 0.762 | ns | 65 | 9 | 0.766 | 0.677 | ns | 0.014 |
| Hi09 | 24 | 10 | 0.848 | 0.542 | ** | 20 | 6 | 0.830 | 0.889 | ns | 21 | 8 | 0.859 | 0.619 | ** | 54 | 10 | 0.862 | 0.630 | ns | 0.016 |
| Hi10 | 24 | 2 | 0.479 | 0.250 | * | 20 | 3 | 0.420 | 0.333 | ns | 21 | 3 | 0.563 | 0.238 | ** | 57 | 3 | 0.503 | 0.263 | ** | 0.004 |
| Hi11 | 24 | 6 | 0.727 | 0.708 | ns | 20 | 6 | 0.755 | 0.800 | ns | 21 | 6 | 0.783 | 0.714 | ns | 65 | 7 | 0.754 | 0.738 | ns | 0.001 |
| Hi13 | 24 | 10 | 0.845 | 0.750 | ns | 20 | 8 | 0.846 | 0.700 | * | 21 | 10 | 0.835 | 0.810 | ns | 65 | 11 | 0.851 | 0.754 | ns | 0.013 |
| Hi15 | 24 | 12 | 0.887 | 0.833 | ns | 20 | 7 | 0.790 | 0.778 | ns | 21 | 7 | 0.711 | 0.667 | ns | 64 | 13 | 0.816 | 0.762 | ns | 0.026 |
| Hi16 | 24 | 6 | 0.617 | 0.542 | ns | 20 | 4 | 0.604 | 0.500 | ns | 21 | 4 | 0.640 | 0.762 | ns | 65 | 6 | 0.620 | 0.600 | ns | -0.002 |
| Mean | 24 | 5.42 | 0.608 | 0.524 | - | 20 | 4.25 | 0.568 | 0.526 | - | 21 | 4.92 | 0.628 | 0.544 | - | 65 | 6.08 | 0.608 | 0.527 | - | 0.008 |

The number of individuals ($N$), number of alleles ($A$), expected heterozygosity ($H_E$), observed heterozygosity ($H_O$), $p$-value for heterozygote deficit ($P$), and $F_{ST}$ among three populations for each locus.

## DISCUSSION

In this study, I successfully analyzed cytochrome *b* gene for genetic diversity and microsatellite loci for population structure of the Korean water deer populations those live in the Korean Peninsula. Koh et al. (2009) previously reported two clades (major and minor clades) of the Korean water deer populations based on control region and cytochrome *b* region. Similarly, our study also determined two clades of the cytochrome *b* sequence from three populations in the Korean Peninsula.

In the past, Korean population could have experienced to be geographically separated into two regional groups forming genetic differences, but at present, it is considered that the Korean water deer populations are a single population.

It is thought to be important to compare with the Chinese water deer as a closely related species. According to previous study, the haplotype diversity and the nucleotide diversity of the Chinese water

deer was 0.923 and 1.318%, respectively (Hu et al., 2006). Compared to the Chinese water deer, the Korean water deer has a relatively low-genetic diversity. Because loss of genetic diversity may decrease the survival and adaptation diversity under the unpredictable environment (Crandall et al., 2000), animal species with low-level of genetic diversity could be encountered the extinction. The Korean Peninsula where the Korean water deer distributed has a topographical characteristic, in that it is artificially isolated area from adjacent North Korea and China. As a result, it is considered that there was no individual exchange or gene flow between Korean water deer population and adjacent water deer populations. Thus, it needs to maintain and increase genetic diversity of the isolated populations within Korean Peninsula by designing corridor or immigrating among populations.

Although the Korean water deer distributed in Korean Peninsula widely, its distribution range and population size is drastically reduced due to poaching, habitat destruction and confliction with

human. According to the recent study of distribution of the Korean water deer, this subspecies is not common in or near large cities, and human population size is significantly related to their occurrence (Kim et al., 2010). In this study, the population structure among 3 populations was not detected. However, there were differences of the genetic diversity among populations. The haplotype and nucleotide diversities of the Northern part were higher than those of the Central and Southern part. Our genetic analyses might be revealed the evidence of the population fragmentation of the Korean water deer. To ameliorate this situation, the inter-population gene flow using corridor could be an effective method.

In conclusion, it is considered that the regional populations of the Korean water deer those are distributed in Korean Peninsula form a singular genetic population structure. Additionally, I might need to prepare the conservation strategy of this species owing to the relatively low genetic diversity.

# GENERAL CONCLUSION

A general pattern of the skull growth of the Korean water deer were presented for each sex. The growth pattern between cranium and mandible was similar in the male and female, respectively. However, each sex showed a distinctive growth pattern. In the case of female, the growth of length factors of the skull was relatively faster than that of male. On the other hand, the growth of breadth factors of the skull in male was relatively faster than that of female. Whereas the fundamental information gained in this study is useful, more precise age group data needed for future study.

The Korean water deer was sexually dimorphic from cranial traits; however, sexual dimorphism was not detected from mandibular traits. The length of the skull of the female was longer than that of male, significantly. This kind of unique sexual dimorphism can be an important feature of this species and a result of evolutionary process, like a type of parental care.

Two subspecies of water deer shares the morphological features from cranial traits. Thirty and 29 measurements from 36 measurements were not significantly different on male and female, respectively. In addition, the result of PCA, morphological distance and cluster analysis using UPGMA (Unweighted Pair Group Method with Arithmetric Mean) and NJ (Neighbor-Joining) argorithn and also supported morphological similarity of two subspecies.

A total of 21 mitochondrial cytochrome *b* gene haplotypes were detected in 3 populations of the Korean water deer within Korean Peninsula. Based on the result of phylogenetic trees using cytochrome *b* region, two distinctive clades were detected. However, genetic distance was not large enough to divide two clades and there was no geographic division within each clade. Analysis using microsatellite markers also showed consistent result with cytochrome *b* gene in that there is no population structure.

The information obtained in these studies regarding the growth pattern of the skull, the sexual dimorphism of the skull, the skull

morphological difference between two subspecies of water deer and phylogenetic status and population structure of the Korean water deer will be useful to understand its morphological and genetic characteristics including behavioral and ecological features, and furthermore to contribute to its conservation and management strategy of this species in China and Korea, probably.

The results of these studies have the possibility to be presented that two subspecies of water deer can be united as one management unit (MU). The concept of a potential 'MU' is as follows: any population that exchanges so few migrants with others as to be genetically distinct from them will normally also be independent of them demographically, at least at the present time. In the literature of commercial fisheries, MUs are often referred to as "stocks," toward which harvesting quotas or other management plans are directed (Avise, 2004). Furthermore, MUs can be identified provisionally by significant differences in allele frequencies at neutral marker loci (e. g. mitochondrial DNA).

Thus, the phylogenetic and molecular genetic characteristics of water deer studied by me and other researcher can support the unified MU of water deer populations from China and Korea. Additionally, study using microsatellite markers can complement this concept of MU, and it will be a basic and fundamental data for water deer populations' management and conservation.

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# 한국산 고라니(*Hydropotes inermis argyropus*)의 머리뼈 계측 및 분자유전학적 연구

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## 국문초록

고라니(*Hydropotes inermis*)는 고라니 속 (genus *Hydropotes*)에 속해있는 유일한 종으로, 전통적으로는 지역적 분포와

털 색깔에 따라서 중국에 서식하는 *H. i. inermis*, 한국에 서식하는 *H. i. argyropus* 2개의 아종으로 분류된다. 그러나, 중국에 서식하는 고라니와는 달리 한국에 서식하는 아종에 대한 연구는 활발하지 않다. 이 논문에서는 고라니 개체군의 머리뼈의 형태적인 특징과 한국 고라니 집단의 유전적인 특성을 조사하였다. 즉, 이 논문의 Chapter I에는 고라니 머리뼈 형태에 관하여, Chapter II에는 유전학적 연구에 관하여 연구하였다.

Chapter I에서는 세 가지 주제를 연구하였다. 첫째, 머리뼈 계측법을 이용하여 한국 고라니의 머리뼈 성장을 분석하였다. 둘째, 머리뼈 계측 분석을 통해서, 한국 고라니의 머리뼈의 성적 이형성을 분석하였다. 셋째, 위턱뼈 계측을 바탕으로 고라니의 두 아종(*H. i. inermis* and *H. i. argyropus*) 사이의 위턱뼈 형태 차이를 분석하였다.

Chapter Ⅱ 에서는 두 가지의 주제로 나누어 연구하였다. 첫째, 고라니의 미토콘드리아 cytochrome *b* gene의 유전적 다양성을 조사하였고, 둘째, 12개의 microsatellite markers를 이용하여 고라니의 개체군 구조를 분석하였다.

계측 분석의 첫째 주제는 한국 고라니의 성장에 있어서 나이와 성별에 따른 크기 변화를 분석하였다. 위턱뼈에서 34개의 계측점 및 아래턱뼈에서 11개의 계측점을 지정하여 계측하였다. 이들 계측점에 관한 통계적인 비교에 따르면, 성별 간에 성장 패턴이 유의하게 차이가 있었다. 수컷의 성장 패턴은 나이 그룹을 통해서 지속적인 성장을 하는 반면, 수컷의 위턱뼈 및 아래턱뼈는 나이 그룹 2-3 단계에서 상대적으로 빠른 성장을 보여주었고, 나이 그룹 3-4 단계에서는 상대적으로 완만한 성장을 보여주었다. 상대성장 분석에서, 성별 간에 5개의 계측점에서 성장의 차이가 있었다. 이번 연구에 따르면, 한국 고라니의 암컷과 수컷은 성장에 있어

서 작은 차이를 가지지만, 비슷한 경향성을 가지는 것으로 생각된다.

계측 분석의 둘째 주제로 한국 고라니의 머리뼈의 성적 이형성에 대한 연구를 실시하였다. 위턱뼈의 데이터만을 이용한 분석에 따르면, 암컷과 수컷의 차이를 잘 보여주었다. 하지만, 아래턱뼈의 데이터만을 이용한 판별분석은 암컷과 수컷을 명확하게 분리하지 못하였다. 두 성별 간의 가장 명확한 차이를 보여준 계측점은 앞니뼈폭(the incisive bone breadth)이었는데, 수컷이 암컷보다 약 12% 정도 더 컸다. 앞니뼈폭(The incisive bone breadth)은 수컷의 송곳니를 반영하는 계측점이다. 이것과 반대로, 대부분의 위턱뼈의 계측점은 암컷이 더 컸는데, 이것은 전체적인 머리뼈의 크기가 수컷보다 암컷이 크다는 것을 의미한다. 일반적으로 동물의 수컷이 암컷보다 크지만, 이러한 고라니의 성적 이형성은 포유류에서 독특한 것으로 생각된다. 이러한 원인으로는 고

라니의 독특한 양육방법 같은 것을 이유로 들 수 있다.

계측 분석의 셋째 주제로, 고라니의 2아종 간의 머리뼈 형태의 차이를 연구하였다. 전통적으로, 2아종으로 분류되어 있지만, 최근의 유전자 연구 결과는 이러한 분류에 대해 의문을 제기하였다. 이러한 분류 체계에 대한 재평가를 위해서, 두 종의 머리뼈 형태 차이 분석을 실시하였다. 통계 분석에 따르면, 두 아종의 계측치는 유의하게 차이가 없었으며, 이것은 두 아종의 개체들은 공통적인 형태적 특징을 공유한다는 것을 의미한다. 두 아종이 각기 다른 위도에 분포하지만, 명확한 형태적 구배는 확인되지 않았고, 이것은 베르그만의 법칙(Bergmann’s rule)이 고라니에 있어서 적용되지 않는다는 것을 의미한다. 판별분석에 따르면, 몇몇 개체들은 양쪽 집단에 의해서 공유되었고, 모든 개체들이 원 집단에 할당된 것은 아니었다. 주성분분석의 결과에 따르면, 두 집단은 75% 이상의 개체들을 공유하였고, 이것은 아종

분류를 위한 “75% 규칙”과 일치하는 것을 생각된다. Neighbor-joining 방법과 Unweighted pair group 방법을 이용한 cluster analyses은 두 아종의 개체들이 서로 혼합되어 있는 결과를 보여주었다. 그리고, 이 결과는 두 아종의 전체적인 형태적인 변이를 보여주지 않는 것을 말한다. 이번 연구의 결과는 유전자 연구 결과가 지적했던 고라니의 분류학적 위치에 대한 재검토와 동일한 결과를 보여주고 있고, 고라니의 두 아종에 대한 분류학적 지위는 재고되어야 할 것으로 생각된다.

넷째 및 다섯째 연구로 고라니의 미토콘드리아 cytochrome *b* 유전자의 1,140 base pair와 12 microsatellite markers를 이용하여 각각 실시되었다. 한국 내 3개 지역에서 51개의 표본을 이용하였는데, 21개의 haplotype이 발견되었고, 전체적인 유전적 다양성은 중국 고라니에 비해 비교적 낮았다. 비록 계통 분석결과가 두 개의 구분된 계통 분기군을 보여

주었지만, 지역적 분기는 확인되지 않았다. Microsatellite variability는 3개의 집단 사이에 유의하게 차이가 있는 것으로 확인되지 않았다 (mean $F_{ST}$ = 0.008), 그리고 이것은 한국 고라니가 유전적으로 집단 구조를 가지고 있지 않는다는 것이다. 한국 고라니는 단일 개체군을 형성하고 있는 것으로 생각된다.

Chapter I과 II의 얻어진 결과들은 고라니의 형태적/유전적인 특성을 이해하는데 매우 유용하고, 나아가 한국과 중국에서 고라니의 보전 및 관리 전략을 수행하기 위해 유용할 것으로 생각된다.

**주요어**: 고라니, 성장 패턴, 성적 이형성, 유전적 다양성, 집단 구조, 머리뼈 형태, cytochrome *b* 유전자, Microsatellite Marker

**학번**: 2008-30992

# SUPPLEMENT

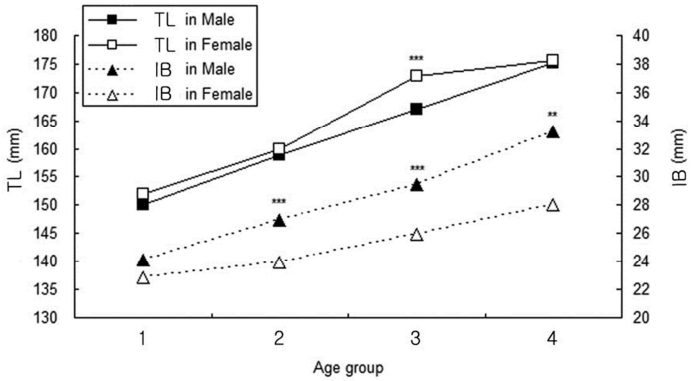


Fig. 18. Growth pattern of TL (left Y-axis) and IB (right Y-axis). Points dotted represent the mean value. Significant difference between male and female: ** $p < 0.01$, *** $p < 0.001$.

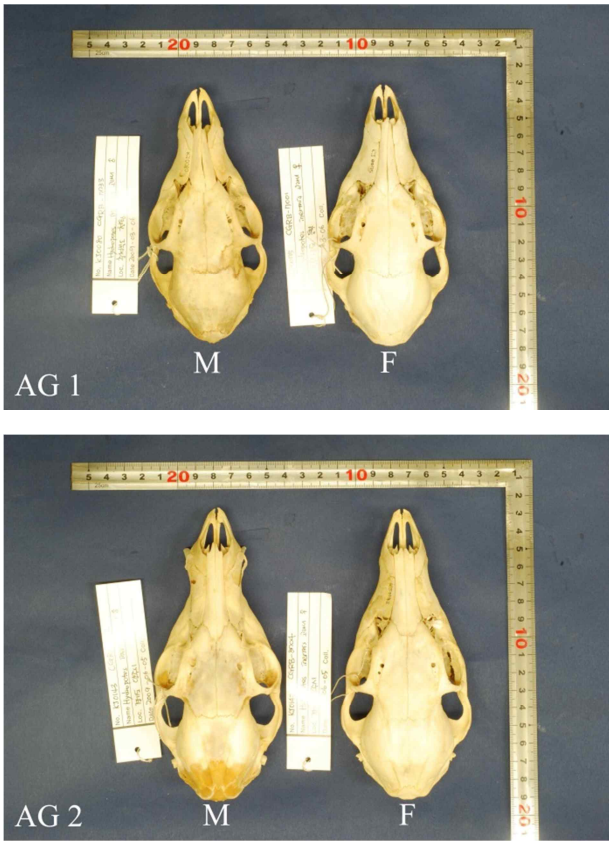


Fig. 19. continued.

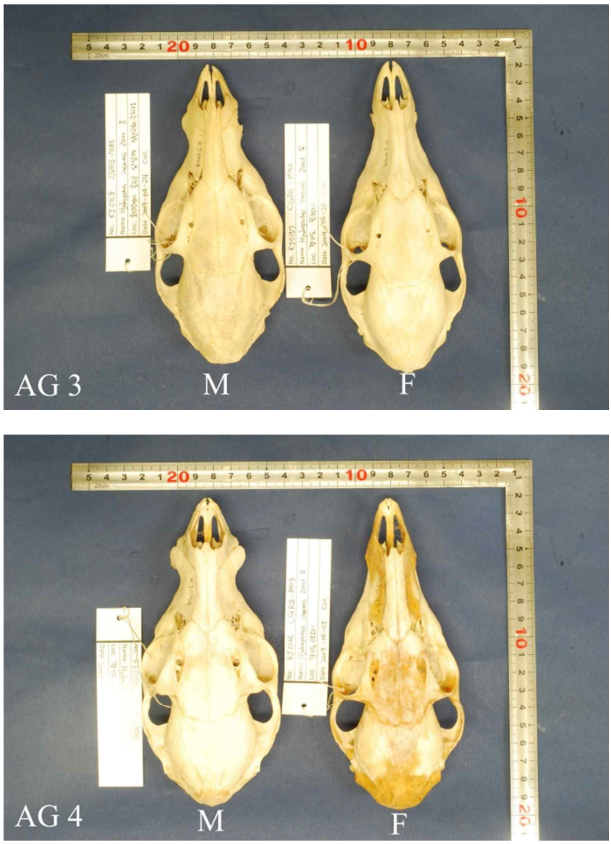


Fig. 19. Comparison of cranium size between male and female. M = male and F = female.

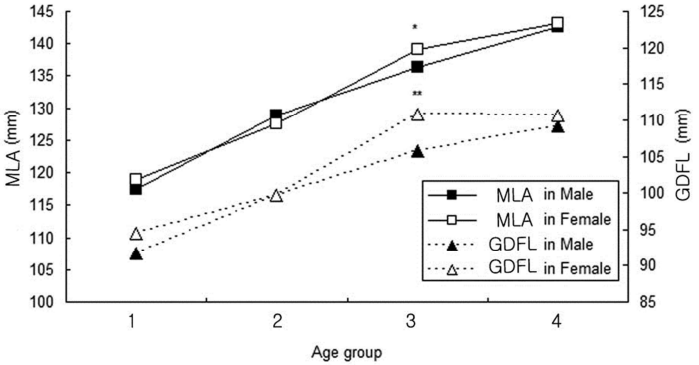


Fig. 20. Growth pattern of MLA (left Y-axis) and GDFL (right Y-axis). Points dotted represent the mean value. Significant difference between male and female: * $p < 0.05$, ** $p < 0.01$.

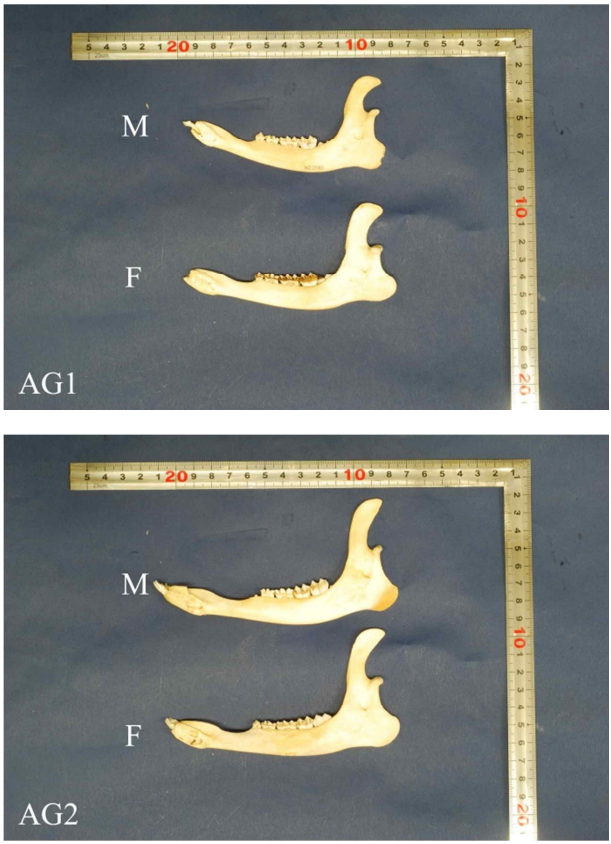


Fig. 21. continued.

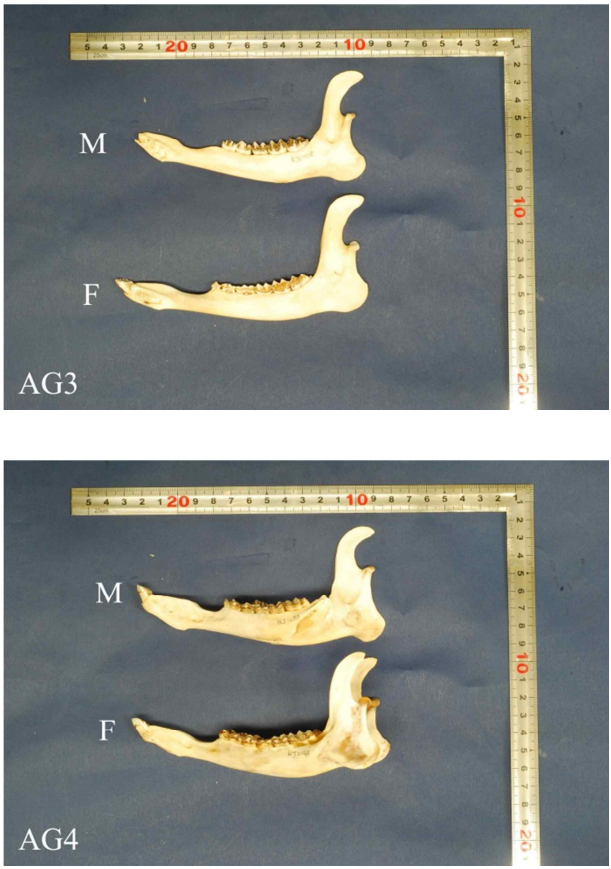


Fig. 21. Comparison of mandible size between male and female. M = male and F = female.

# ACKNOWLEDGEMENTS